Shadowy futures – The effect of turbidity on yellow-eyed mullet (*Aldrichetta forsteri*)

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Abstract:

Worldwide turbidity is a huge concern for the health of aquatic ecosystems. Human activities on the land such as construction, deforestation, agriculture, and mining all have impacts on the amount of particulate solids that enter the world's waterways. These particulate solids can pose a number of risks to aquatic life, but primary among them is the turbidity that they create in the water column. The way suspended solids interact with light creates cloudiness in the water which interferes with the vision, and visually mediated behaviours of aquatic organisms, particularly fish. The Avon-Heathcote estuary of Christchurch, New Zealand, is one such body of water that is subject to tremendous variation in turbidity, no doubt exacerbated by the destruction of Christchurch in the 2010 and 2011 earthquakes, as well as the subsequent ongoing rebuild. The yellow eyed mullet, *Aldrichetta Forsteri*, is one species that is common with the estuary, and uses it as a habitat for breeding. Though very common throughout New Zealand, and even a part of the catch of commercial fisheries, the yellow eyed mullet is a largely unstudied organism, with virtually no published scientific enquiry based on the species. The present work assesses how several behaviours of the yellow eyed mullet are effected by acute turbidity at 10, 50, 90, 130 and 170 NTU, finding that: 1) The optomotor response of mullet to 2.5 mm stripes drops to insignificant levels between 10 and 50 NTU, 2) The swimming activity of the yellow eyed mullet is highest at 10 NTU and drops to a significantly lower level at higher turbidities, 3) The grouping behaviour of small groups of yellow eyed mullet are unchanged by increasing turbidity levels, 4) that yellow eyed mullet do not exhibit significantly different behavioural response to a simulated predator at any of the tested turbidities, and 5) that yellow eyed mullet to do significantly alter their oxygen consumption during exposure to the turbidities in an increasing series. The results presented in these studies indicate that turbidites above 50 NTU pose a significant risk to the lifestyle of the yellow eyed mullet, potentially impacting their ability to perceive their surroundings, feed, school, and avoid predation. Future work has a lot of ground to cover to more precisely determine the relationship between yellow eyed mullet behaviour and physiology, and the turbidity of their environment. In particular, future work should focus more closely on the turbidities between 10 and 50 NTU, as well as looking to field work to see what the predominant predators of the mullet are, and specifically whether turbidity increases or decreases the risk of mullet being subject to avian predation. There is also considerable scope for studies on the effects of chronic turbidity upon mullet, which will add understand to the predicament of escalating turbidity and its effects upon this common and yet mysterious native fish.

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CONTENTS

1 INTRODUCTION:

1.1 TURBIDITY

Turbidity is a measure of the cloudiness of a fluid created by large numbers of small suspended particles. These particles can cause light passing through the fluid to scatter, invalidating the eyes assumption of rectilinear propagation of light and causing haziness of an image. Here, the fluid in question is the water in which aquatic organisms live, and the suspended particles those that are often found in these environments. In an aquatic environment, this suspended material would typically consist of soil particles, and small organisms such as algae and plankton, and often, due to human activity, pollutants as well.

There are numerous units used for measuring turbidity, however the one used here is Nephelometric Turbidity Units (NTU). Nephelometric turbidity is an index of the scattering of light as caused by suspended matter. Because the majority of the impacts caused by turbidity are related to the attenuation of light, and different materials interact with light in different ways, directly measuring light scattering is often more relevant than measuring the mass concentration of suspended matter.

Though turbidity is a natural aspect of aquatic environments, anthropogenic activities can cause large changes from the norm, increasing the upper limits of turbidity that these environments reach, and the frequency with which they reach these levels. Hayward et al. (2006) describes the worldwide growth in human population causing dramatic changes to freshwater and marine ecosystems. The intensive activities of human settlement often contribute foreign pollutants to nearby environments, as well as exaggerated quantities of otherwise naturally occurring sediments. These activities include, but are not limited to, river damming, deforestation, agriculture, construction, and sewer works. Deforestation is the main driver of erosion, with eroded earth entering streams and rivers at tremendous rates, the turbidity of these waterways, and the estuaries and coasts where they lead increasing as a result. The activities within human settlements, such as construction, contribute particulate matter, much of which is not naturally occurring, to water ways via drains that lead to the same waterways. And finally, sewer works and agriculture are both responsible for the introduction of organic nutrients to the environment, resulting in algae blooms and other eutrophication.

The problems created by unnaturally elevated turbidity are well documented in water systems all over the world, and are considered to be one of the largest drivers of the degradation of these

environments. Often the primary source of the turbidity found in lakes, estuaries, and coastal areas is human induced processes occurring upstream around freshwater waterways. Large scale clearing of forests weakens the soils where they once stood. Without roots holding the earth in place, rain and wind causes significant erosion, with the loose soils being washed downhill towards waterways such as streams. Erosion is so linked to turbidity, that the turbidity of a location in a stream or river is often used as a metric of the erosion that is occurring further upstream (Sun et al. 2001).

The damming of rivers can have an effect on the turbidity of a waterway also. While the construction of said dams undoubtedly causes elevated turbidity from earthworks, concrete dust etc., an already constructed dam often acts as a catchment for sediments (Neary et al. 2010). The relatively stagnant water held behind a dam wall allows these sediments to drop out of suspension, however in doing so, they slowly reduce the storage capacity of the dam. To this end, when a dam is opened, a great deal of particulate matter can be dumped into a waterway all at once. Although the resulting elevated turbidity is relatively short lived, the effects can be dramatic, even directly resulting in fish mortality (Baoligao et al. 2016). Neary et al. (2010) investigated the effects of harvesting trees in agricultural streamside management zones. It found that if performed carefully, trees could be removed from these zones without significantly influencing the turbidity of the stream, but that the proximity of cattle and a road to the stream proved to be much larger factors in influencing turbidity. Cattle contribute to the turbidity of streams and rivers adjacent to their paddocks in a few different ways. As summarised in Scrimgeour and Kendall (2003) cattle can destabilise stream/river banks with the mechanical action of their hooves, eat riparian vegetation which further destabilises banks, directly contribute organic particulate matter to the water in the form of manure, and promote eutrophication with the introduction of nitrogenous compounds, again from their waste.

Estuaries are a type of water body which can be heavily impacted by anthropogenic turbidity. A number of processes, many natural, contribute to the extreme variability of turbidity that is characteristic of estuarine environments. The tidal action at the estuary mouth and the outflow from rivers and streams, in particular, are largely responsible for the high turbidity that is a common characteristic of estuarine environments (Benfield and Minello, 1996), however wind mixing and the presence or absence of silt traps (such as reed beds) play an important role also (Bruton 1985). Some estuaries are used as transportation routes for boats, and in these cases the routes that the boats use are dredged to remove any obstructions. This act of dredging re-suspends sediment from the floor of the estuary, and too contributes to elevating the turbidity of the estuary in question (Wilber and Clarke 2001).

A study by Wilkinson (1999) assigns increased sedimentation resulting from human activities to be among the most major risks to coral reefs internationally. In the cases of coastal waters, or on those above a continental shelf, where corals are often found, the sediments washed out to sea rarely settle as wave action continually re-suspends particles, making elevated turbidity, and the issues it creates, to be a constant problem. Again, just like with estuaries and rivers, increasing turbidity in coastal waters is driven by the same human activities occurring further inland.

1.2 EFFECTS OF TURBIDITY ON FISH

Elevated turbidity, as a result of human activity, is a widespread phenomenon that effects a large range of ecosystems. But what are the consequences of elevated turbidity? As already mentioned, sometimes the particulate matter responsible for turbidity can be sorbed with chemical pollutants, or be toxic in their own right. However this is beyond the scope of this thesis, and instead the focus is on the physical effects of turbidity that even inert particulate materials can inflict upon an ecosystem. These effects are numerous, and typically manifest themselves most obviously at very high levels of turbidity. Bruton (1985) describes the main effects as 1) shading by the suspendoids resulting in the reduction of light penetration into the water, reduced photosynthesis, and therefore a reduction in plant biomass and food availability, 2) reduced visibility of pelagic food items, 3) Reduced visibility of benthic food due to smothering, 4) clogging and/or abrasion of respiratory apparatus such as gills, and 5) reduced risk from aerial predators. Though not a comprehensive list, these, for most aquatic environments, would be the main considerations on the impact of elevated turbidity.

Although there are some cases where turbidity can prove to be beneficial to a fish species, at least in some aspects, largely most of the effects are negative. In studies of species abundance, turbidity is often found to be negatively correlated with the number of species present in a given area. For example, in New Zealand, much of the turbidity research that has been conducted has been on species of freshwater fish. Richardson and Jowett (2002) undertook an investigation into species abundance in streams of the North Island's East Cape. Of the 38 streams surveyed in this area, many of them were subject to high turbidity loads which are exacerbated by the fact that much of the naturally forested land has been cleared for agriculture. They found that in low sediment streams they were able to identify up to as many as 9 different fish species, but in equivalent streams with high turbidity, the number of species dropped as low as two.

In a similar study based on data from estuaries in the San Antonio Bays and Aransas areas of Texas, Lunt and Smee (2014) found that turbidity was again negatively correlated with fish species abundance. Estuaries with turbidities lower than 30 NTU were found to have the highest numbers of fish species, and interestingly high turbidity estuaries (>30 NTU) were found to have more mud crab species – a family which typically doesn't rely on vision to find and capture prey.

Fish being detrimentally effected by turbidity

Turbidity has been demonstrated to affect many different species of fish in a wide range of ways. Primarily, turbidity is a visual feature of an environment, and thus interferes with behaviours and aspects of physiology which are visually mediated. This interference can manifest itself in a number

of ways, including influencing sexual selection. In Seehausen et al. (1997) turbidity has been shown to influence mate choices in cichlids. Multiple cichlid species can reside in the same lake and maintain species boundaries despite being perfectly capable of producing fertile offspring via interbreeding. Cichlid mate choice is determined on the basis of colouration, and strong assortive mating can quickly lead to the creation of different species isolated by mate choice. Turbidity disrupts the visual cues typically used in this kind of sexual selection, particularly colour, and can effectively remove the barriers between otherwise compatible cichlid species. Reflecting this, Seehausen et al. (1997) reports that cichlid populations found in areas which have become turbid as a result of eutrophication possess fewer colour morphs, are overall duller in colour, and have lower species diversity.

Similarly, Engström-Öst and Candolin (2007) have found that turbidity affects sexual displays in sticklebacks, *Gasterosteus aculeatus*. Stickleback males rely on their appearances and courtship displays to attract females. The better their displays, the more often females will visit and the longer each visit will last. The study found that under turbid conditions a male stickleback needed to put in considerably more effort into courtship displays to attract the same amount of attention that they would otherwise receive in clear water. Engström-Öst and Candolin hypothesised that under increased turbidity, sexual selection would favour courtship displays over bright colouration, which would incur higher energy costs and potentially increase predation risk. Thus turbidity clearly can very directly affect sexual selection and steer the evolution of the species it effects.

In another example of turbidity changing the dynamics of sexual selection, Jarvenpaa and Lindstrom (2004) have shown a significant reduction in the intensity of sexual selection in sand gobies, *Pomatoschistus minutus*, living in turbid water. Sand gobies utilise a resource-defence mating system where males are the only sex responsible for parental care. In clear water, large sand goby males tend to attract more females and convince them to lay eggs in their territories, while smaller males may not successfully mate at all. The study showed that in turbid water mating success was far more evenly divided over all males, suggesting that the turbidity of the water impaired the ability of the females to make informed mate choices.

Of course the characteristic light scattering that occurs in turbid waters is merely a symptom of the presence of suspended particulate matter in the water. Being a physical substance, suspended particulate matter can interact directly with aquatic organisms as well. As pointed out in Bruton (1985) fine particulate matter suspended in the water column can cause clogging and abrasion of the gills of fish. Lowe *et al.* (2015) investigated the effects of long term exposure to increased turbidity on snapper, *Pagrus auratus*, paying particular attention to the effects on respiration-related features

of the snappers' behaviour and morphology. Short term exposure to elevated turbidity (from <10 – 160 NTU) unsurprisingly found a decrease in foraging success, and month long exposure led to higher levels of gill ventilation, gill deformation in the form of fused lamellae and epithelial hyperplasia, weight loss, and death. In the field, turbidity was found to be negatively correlated with snapper catch, positively correlated with gill deformation and gill parasites, and catches indicated that in more turbid environments, snapper were prey switching to benthic items, away from pelagic zooplankton. Although no gill deformation was found, Reid et al. (2003) also showed that in the short term (30 hours) human-caused turbidity induced a higher respiration rate in rainbow trout, *Oncorhynchus mykiss*. The effects of turbidity on fish respiration are discussed in more detail in chapter 5.

Many fish have a sophisticated ability to learn to recognise other species as predators and non-predators, and can often generalise from these learnings and decide if novel fish presents a threat or not. Fat head minnows, *Pimephales promelas*, are one such fish that possess this ability. However, the learning capabilities of the minnows are dramatically reduced when said learning occurs in turbid water. Chivers et al. (2013) demonstrates this by presenting minnows with a predator and a chemical alarm cue simultaneously. This is a very efficient way to learn to identify a new threat, as it only takes one encounter like this for the minnows to show a predator response when encountering the predator from then onwards. However, when the initial learning experience with the chemical cue was performed in turbid water, the minnows' processes of learning to identify a predator and to generalise from that predator were severely impaired. Minnows taught to recognise one type of trout as a predator in clear water could then generalise their experiences, showing a strong fear response to another type of trout, and a mild one to an entirely novel fish species, perch. However those that had learnt to fear the trout in turbid water, did not manage to learn as well from this experience, showing a lower response to this same trout later (compared to the clear water learner), and showing only a weak response to the novel trout indicating that they had not been able to extrapolate the experience.

One of the most significant impacts that increased turbidity has upon fish is the impact it has on their ability to feed as a result of impaired vision. As a New Zealand based example, Greer et al. (2015) shows that increased turbidity decreases the feeding rates of brown trout. The authors hypothesised two ways in which turbidity can impact feeding. The first was that physiological stress brought on by turbidity could reduce the tendency of the trout to express feeding behaviours. The second is that in conditions of reduced visibility, the trout, as has been shown in many fish species (Bruton 1985; Leahy et al. 2011), become less active, conserving energy at a time where feeding success would be lower due to a reduced ability to detect and pursue prey in the turbid water. Given

that their trout showed no changes in their respiratory performance at any of the tested turbidities, it was deemed that the latter was in fact the case.

Fish coping with turbidity

The more visually orientated an organism is, the more it might be expected that it would be susceptible to the effects of turbidity. However, it would be disingenuous to omit reference to the fact that some organisms possess adaptive traits to help them mitigate the effects of turbidity, or even take advantage of them; either allowing them, within certain limits, to operate near or even above normal levels in turbid conditions. For example, guppies have been demonstrated to have developmental plasticity to help them overcome turbidity (Ehlman et al. 2015). In this study guppies, *Poecilia reticulate*, were reared in either turbid (40-60 NTU) or clear water. Adult guppies which had been raised in clear water followed the trend typically seen in activity experiments where they reduced activity when the turbidity of their tank was increased. Guppies raised in turbid conditions however demonstrated higher levels of activity in turbid conditions than in clear ones. This difference was put down to the opsin gene expression in the guppies. Guppies reared in the turbid conditions expressed more long-wavelength sensitive opsins, which is significant as long-wavelength sensitivity is important in motion detection and long wavelength light penetrates turbid waters more easily. Therefore the shift in gene expression allowed the guppies to salvage their motion detection ability in suboptimal conditions.

Deprived of the opportunity to alter gene expression during development, some species rely on other senses to help compensate for their impaired sight. In elevated turbidities, fat head minnows reacted more strongly to chemical cues than they otherwise would when in clear water (Hartman and Abrahams, 2000). Being able to switch which sense mediates a behaviour is a highly adaptive trait in variable environments.

While increased turbidity means that some fish have a harder time locating prey items, this also must mean that turbid conditions must confer an advantage to some fish when it comes to hiding from their own predators. For example, pike larvae, *Esox lucius*, reduce anti-predator behaviours in turbid conditions, indicating that turbidity provides them with cover, helping hide them from the view of predators (Lehtiniemi et al. 2005).

In a final example of turbidity bestowing an advantage to fish, Jarvenpaa and Lindstrom (2011) reports that under turbid conditions sand goby males dedicated less time to nest care, strayed further from their nests, and yet those nests had higher rates of egg survival than compared to trials in clear water. The reasons behind this are unclear as it is suggested that in the wild, high turbidity is

synonymous with lower population density of sand gobies. Due to lower competition from other males, sand goby males would be expected to put more effort into their nests as paternity would be more certain. As higher parental input was not observed, it remains unclear why turbidity was correlated with better egg survival. It is of note that in this study, there was no predator presence. The behaviour of sand goby males straying further from their nests in turbid conditions is possible to aid them in finding females, but under real world conditions such behaviours may expose their nests to higher predation rates.

Covered in more detail in later chapters are the ways that acute increases in turbidity effects visual acuity (chapter 3), the activity levels of fish (chapter 4), and the respiration of fish (chapter 5).

1.3 YELLOW-EYED MULLET (*ALDRICHETTA FORSTERI*)

Yellow-eyed mullet, *Aldrichetta forsteri*, (referred to hence forth as just yellow-eyed mullet) are found all around New Zealand's coast and make up a large and dominant part of its estuarine fish fauna (Curtis and Shima, 2005). Despite its abundance, the yellow-eyed mullet is an understudied organism with very little published information on its biology or the extent of commercial landings of this species (Ministry for Primary Industries, 2014). Yellow-eyed mullet are a member of the Mugilidae family, making them true mullets. One of two mullet species in New Zealand (the other being the grey mullet), their range within New Zealand extends from North Cape to Stewart Island. Yellow-eyed mullet are typically a schooling species (starting schooling when still in the larval stage, at sizes as small as 4mm (Masuda and Tsukamoto, 1998)), and are common along the coast, in estuaries, and in lower river systems of New Zealand. Adults, which can get up to 7 years old, tend to prefer marine environments while juveniles are known to be seen in freshwater, feeding on algae (Ministry for Primary Industries, 2014). An opportunistic feeder, the diet of the yellow-eyed mullet includes algae, crustaceans, diatoms, molluscs, insect larvae, small fish, polychaetes, coelenterates, fish eggs and detritus (Ministry for Primary Industries, 2014). It is this diverse diet that means yellow-eyed mullet must stay very active so that they are constantly searching for food. It is precisely this activity that will be a focus of this chapter 3. Generally egg development begins in July and maturity occurs in late December. Spawning occurs during summer (December to mid-March) (Ministry for Primary Industries, 2014). In the Avon-Heathcote estuary, Christchurch, New Zealand (where fish were gathered from – more on this is in Chapter 2), spawning likely occurs twice a year, peaking in summer and then again winter, as mature females are found in June-July and November to February (Webb, 1973). Yellow-eyed mullet are pelagic spawners, meaning ova and sperm are simply ejected into the water for random fertilisation (Webb, 1973).

The yellow-eyed mullet display a discrepancy in relative growth rates over latitudinal gradients. Curtis and Shima (2005) found that within each of the North and the South island (but not across), females tended to grow faster in the north of each island, with the sexes growing at similar rates, or with the males faster, in the south of each. The paper suggests a few different reasons for why this may be the case, including a relative increase in costs of reproduction at higher latitudes, or conditions that favour sexual dimorphism such as sexual or natural selection.

Yellow-eyed mullet were added to the quota management system in 1998 with a total NZ quota of 56 tonnes/year (increasing to 68 ton in 2001). Since the quota has been introduced, commercial landings have never exceeded those of 1999 – 44 tonnes, although the largest catch (pre-quota) was 68 tonnes in 1986 (Ministry for Primary Industries 2014). Yellow-eyed mullet are often colloquially

referred to as sprat, herring, or just mullet (not differentiating between grey and yellow-eyed mullet). Due to inconsistencies in fishery labelling, there is potential for misreporting in the amount landed each year (Ministry for Primary Industries, 2014). In the mid 1980's, landings increased, reflecting more fishing in the Auckland area to meet greater demand for yellow-eyed mullet. The average catch from 2009-2014 has been 27 tonnes, only 1 ton lower than the longer-term 30 year average (Ministry for Primary Industries, 2014).

1.4 AVON-HEATHCOTE ESTUARY

Yellow-eyed mullet are very common within the Avon-Heathcote estuary of Canterbury, New Zealand (43.5455° S, 172.7293° E). The Avon-Heathcote estuary is fed by the Avon and Heathcote rivers, and is connected to the ocean by a short inlet. Due to the natural interactions between these bodies of water, the estuary is subject to fluctuating levels of turbidity. Also contributing to these changing turbidity levels is anthropogenic activity. Like other places all over the world, New Zealand water bodies have been subject to increased sediment delivery as a result of human induced disturbances, such as land clearing and agriculture (Hughes et al. 2012) – the Avon-Heathcote estuary is no exception. Starting with the beginnings of Christchurch's urbanisation in the 1850's, the increasing density of the human activity surrounding the estuary and its associated waterways has further contributed to changeable nature of the estuaries turbidity. Activity that contributes to the growing turbidity includes, but is not limited to, earthworks, construction, sewage discharge, vegetation clearance and logging, stock farming, and dredging (Deely, 1991). In particular, since the September 2010 and February 2011 Christchurch earthquakes, this problem has only intensified (Zeldis et al. 2011). The substantial amount of construction and overhauling of drainage infrastructure in the local area is very likely increasing the amount of sedimentation being deposited in the estuary. This is a serious cause for concern, and as follows, could have huge impact on the local fauna and flora, such as the yellow-eyed mullet.

The Canterbury Regional Council (Environment Canterbury) tracks a number of water quality parameters of the estuary. Approximately once per month, the estuary is sampled from 9 different locations at the shore. Along with the time, date, and sampling location, 7 characteristics of the estuary are measured: total suspended solids, turbidity, water temperature, dissolved oxygen, dissolved oxygen saturation, salinity, and how much rain had occurred before the sample was taken. Sampling from these locations show that considerable variation of turbidity is a regular occurrence within the estuary. The turbidity data shows variation at every site, though the extent and frequency of this variation varies between them. Seen in figure 1, the single highest turbidity measurement, 360 NTU, was made on the east side of the estuary, at the end of Penguin Street, (approximately -43.546232, 172.744110). Although, this point seems to be an outlier, other examples of high turbidity can be seen at other sites, such as Sandy point (approximately -43.546369, 172.713755) (figure 2). This natural range of turbidities was used to inform experimental decisions as explained in General Experimental Methods (Chapter 2).

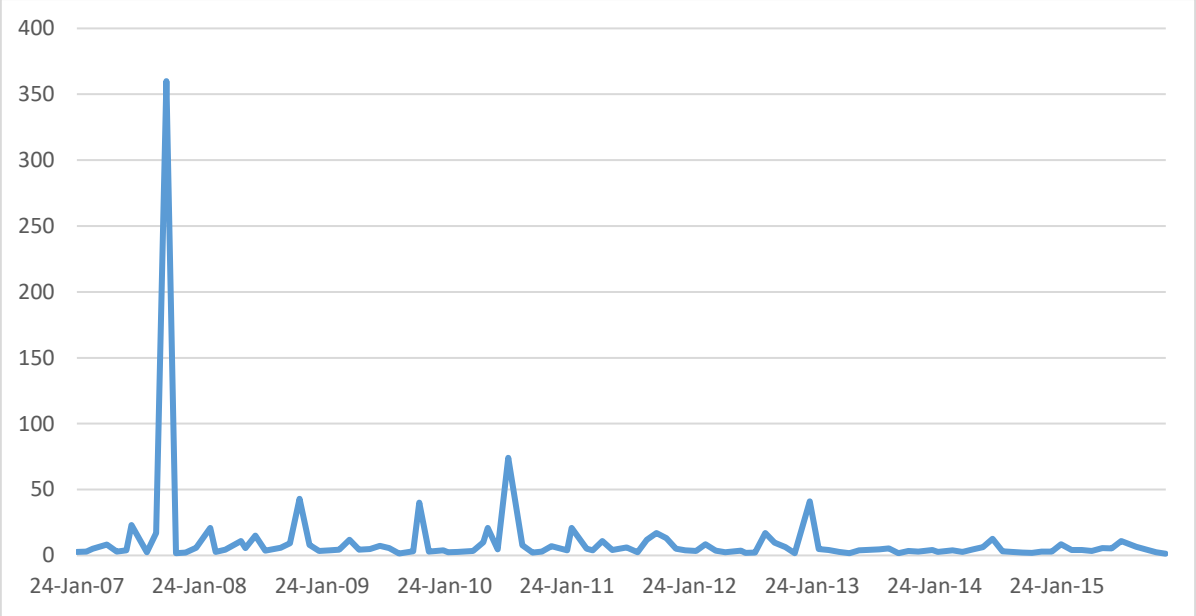

Figure 1 Turbidity sampling at Penguin Street site of the Avon-Heathcote estuary as gathered by the Canterbury Regional Council

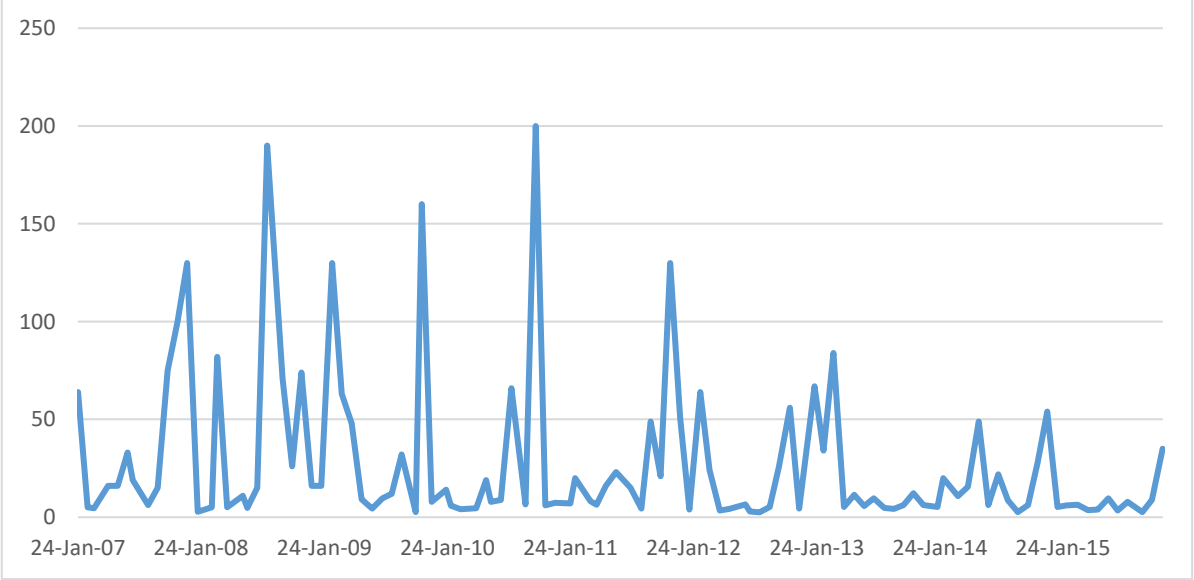

Figure 2 Turbidity sampling at Sandy point site of the Avon-Heathcote estuary as gathered by the Canterbury Regional Council

2 GENERAL EXPERIMENTAL METHODS

Yellow-eyed mullet were caught in the Avon-Heathcote estuary outside of Christchurch, New Zealand (-43.545500, 172.729300). The mullet were caught using fishing rods, mostly from a boat over the channel, and to a lesser extent, from the rocks on the edge of the estuary. To minimise stress and damage to the fish, small hooks were used, the barbs of which were blunted with a pair of pliers. This minimised the amount of time the fish were handled, and disposable latex gloves were worn when handling fish to protect their skin and scales. Once the hooks were removed from their mouths, the fish were placed into an on-board tank which was filled with fresh estuary water and contained an air bubbler. This water had a very small dose (5ml/1000L) of "AQUI-S" added to it in order to sedate the fish, reducing stress, and preventing them from injuring themselves by swimming into the sides of the tank. Fishing trips were brief, generally lasting no more than 90 minutes. Once the boat had returned to shore, the fish were transported into a large insulated bin, again full of estuary water and fitted with an air bubbler, and loaded into the back of a vehicle. The fish were then transported to the University of Canterbury, New Zealand (-43.523500, 172.583900), and transferred to a home tank. The home tank was plastic and was circular in shape, measuring 1.6 metres in diameter. The seawater within the tank was maintained at 15˚C, with a 12 L: 12 D light cycle. Fish were fed to satiation on a diet mainly comprising green-lip mussels (*Perna canaliculus*) and fish pellets. Water changes were regularly administered. All experiments had animal ethics approval from University of Canterbury animal ethics committee (2014/23R).

In order to protect the animals from harm when weighing and measuring them, they were sedated with a low dose (50 mg L^{-1}) of MS222 Tricaine Methanesulphonate. They were measured from nose tip, to the furthest tail tip.

Porcelain dust was used, where necessary, to adjust turbidity levels. In order to improve the mixing of the porcelain duct into the water, first the porcelain dust was mixed into a small amount of seawater, creating a thick, highly concentrated mixture. This mixture was then added into the experimental tank. Porcelain dust was added to the tank at approximately 0.005g L^{-1} NTU^{-1}. A turbidity reader (Hach, 2100P Turbidimeter) was used to measure turbidity and ensure maintenance within 10NTU of the desired level for the duration of the experiment.

Turbidities selected for use in the experiments were based on levels which occurred normally in the Avon-Heathcote estuary (figures 1 and 2), limited to 170 NTU as preliminary testing showed higher turbidities proved too difficult to sustain for the duration necessary to complete the experiments.

3 EFFECT OF TURBIDITY ON THE VISUAL ACUITY OF YELLOW-EYED MULLET (*ALDRICHETTA FORSTERI*)

3.1 INTRODUCTION

Visual acuity is a measure of the ability of an organism to resolve its surroundings. Vision is a vital sense for an enormous number of visually mediated behaviours physiologic systems, playing a crucial role in helping an animal stay alive and reproduce. The effects that turbidity has on visually related behaviours and physiological systems of fish are wide ranging and well documented. In New Zealand, much of the work undertaken to assess the effects of turbidity has been performed with regards to freshwater ecosystems, particularly with regard to the Galaxias family (Ryan, 1991; Boubée et al. 1997; Rowe and Dean, 1998; Rowe et al. 2000). These works reveal that turbidity affects some species (freshwater ones at least) significantly more than others, even when they are closely related. There are also a number of experimental methods which can be used to gauge these effects. The following describes some studies that demonstrate the potential detrimental effects of elevated turbidity on the vision of marine fish, but it is of note that some estuarine fish seem to be well adapted to high turbidity, with it having little to no effect on their ability to forage (Grecay and Targett, 1996; Pekcan-Hekim and Horppila, 2007).

FEEDING

Turbidity has a detrimental effect on the ability of fish to perceive fine details and surroundings, and can therefore make finding and catching food items difficult. The challenge of finding food in a turbid environment specifically affects species which feed on larger food items, such as piscivorous fish, as those feeding on plankton are typically less reliant on vision when seeking their prey. This is because plankton are more difficult to see than fish under any set of visual conditions (De Robertis et al. 2003). Instead, plankton-feeding fish rely more on tactile and olfactory cues which are not disrupted by turbidity, such as the Antarctic fish, *Pagothenia borchgrevinki*, which eats plankton even in over the dark winters near the South Pole (Montgomery et al. 1988). Benfield and Minello (1996) undertook a pair of experiments, the first of which aimed to determine the effects of turbidity on feeding rates. To assess feeding rates, an experiment was designed to see how a turbid water treatment (100 NTU), a shaded treatment (to control for the decrease in light intensity caused by turbidity) and an unaltered control treatment would compare in how they affected the feeding rate of *Fundulus grandis*, the gulf killifish. Fish in the turbid treatment averaged 6.8 shrimp consumed in two hours, while the fish in the shaded and control treatments enjoyed consumption rates of 17.6 and 17.2 shrimp per 2 hours respectively. These results indicate that turbidity has a strong effect on the ability of the killifish to consume prey, and also that this problem is a result of

light scattering due to particles in the water and not a net decrease in light intensity due to shading from the suspended matter.

Lowe et al. (2015), examining juvenile *Pagrus auratus*, snapper, made similar findings. Groups of wild caught juvenile snapper were starved for 24 hours and then placed in tanks of varying turbidity levels (<10, 20, 40, 80 and 160 NTU) along with mysid to prey upon. After 30 minutes of feeding, the snapper were removed and the remaining mysids counted in order to determine how many had been consumed. Elevated turbidities were found to significantly impact the feeding success of the snapper, with 77% of mysids consumed at >10 NTU and only 8% consumed at 160 NTU.

Utne-Palm (2004) found that Atlantic herring larvae, *Clupea harengus*, also demonstrated a lower attack-rate on prey items with elevating turbidity (0, 35, 80 JTU). Larvae of a range of sizes were tested, with the larger larvae having higher attacks rates than the smaller larvae. This relationship was expected as the visually acuity of larvae improves with development. The only exception to the trend of decreasing attack rate with increasing turbidity was that the very smallest of larvae (20mm) showed their highest attack rate at 35 NTU instead of 0. The author presents the idea that intermediate levels of turbidity may increase the contrast between prey items and the background, and that also turbidity may provide larvae with enough cover to engage in risky behaviour such as hunting. To this end, intermediate turbidity may provide the larvae with some advantages to counteract the disadvantages that it typically bestows upon fish.

In a field experiment, Lunt and Smee (2014) tied crabs, *Panopeus herbstii*, in a bay and observed predation rates and the predators responsible for the predation. Under higher turbidity conditions, they found that a lower proportion of crabs were eaten by fish, suggesting that the fish in the region avoided areas of elevated turbidity, struggled to find the crabs in the higher turbidity, or both. Other crabs however, were responsible for more of the predation upon the tethered crabs under turbid conditions than they were under clear ones. This is likely because as crabs use olfactory cues to find food instead of visual ones, they are not effected by turbidity in this way. Lunt and Smee (2015) supports this further, with a lab study comparing the feeding rates of chemosensory predators with visual ones on two different types of prey under a range of turbidities. The prey items selected for the experiment represented visually orientated, and chemosensory orientated prey. Pinfish, *Langodon rhomboides*, a visual predator, consumed significantly less prey under turbid conditions. The feeding rate of the chemosensory predator, the blue crab, *Callinectes sapidus*, was completely unaffected by the turbidity level, showing that turbidity has greater effect on visual animals (typically fish) compared to others.

At only moderate levels of turbidity, other factors can come into play that reduce the impact that turbidity has upon the organism. Juvenile perch, *Perca fluviatile*, a visual predator which relies on clear water good lighting conditions, suffer no significant impact to mysid shrimp feeding rates with turbidites in the range of 1 to 30 NTU (Granqvist and Mattila 2004). This indicates that other factors may be assisting the perch in maintaining their usual feeding rates, such as increased activity of the perch, reduced anti-predator behaviour of the prey, or altered contrast of the prey and the background.

REACTIVE DISTANCE

In the second experiment conducted in Benfield and Minello (1996), humans, *Homo sapiens,* looked through a specially designed tank in an effort to detect an object inside of it in order to give an indication of reactive distance. Reactive distance is defined as either a) the distance at which an organism can detect an object (the definition used for this experiment), or b) the distance at which an organism will react to an object. This experiment was performed at a range of turbidities and it was found that increasing turbidity had a detrimental effect on reactive distance. The effect of adding more particulate matter was diminishing though, with the greatest decline of reactive distance occurring as turbidity increased from low to moderate levels. Although this experiment used human subjects, this is defendable due to the inherent complications in trying to identify when a fish is able to first perceive an object. Benfield and Minello (1996) also maintain that while visual acuity varies between humans and fish species, the shape of the relationship between reactive distance and turbidity is shared by humans and a variety of other fish species. Together these results illustrate that turbidity is a factor which strongly affects visual acuity, and therefore predatory success due to a reduced ability to detect prey items at range.

PREDATOR AVOIDANCE

Turbidity can also hamper efforts of fish to avoid predation. Visual cues are very reliable in space and time, but are hampered by visual obstructions such as turbidity or habitat complexity (Leahy et al., 2011). Some fish, such as the damselfish, will become more reliant on chemical cues in the absence of visual information. The fish in this study increased their cautiousness (an anti-predator behaviour) in turbid environments (4.5, 8.8 and 24 NTU), but this paper is only able to speculate on whether this was enough to counteract the other negative effects of turbidity on the species' feeding rate and risk of predation. These other negative effects include reduced ability to detect the position of predators, if they are currently present, or just have been in the past, or if another fish is indeed a predator at all. There are also other detrimental effects that turbidity inflicts on fish vision, such as those seen to affect the fat head minnow (Ferrari et al., 2010). In this study, minnows were taught to recognise the sight of predatory brown trout. They were then tested for their response to a brown

trout stimulus, a rainbow trout stimulus or a yellow perch stimulus (not a predator of minnows) in one of two environments: turbid and clear water. Minnows in clear water reacted strongly to the brown trout stimulus and generalised this response to the rainbow trout as well. In turbid conditions the response to the brown trout was weaker and no anti-predator responses were recorded towards the rainbow trout. This demonstrates that turbidity severely affects the amount of visual information a fish receives, which in this case eliminates their ability to generalise from known predators to novel ones.

METHODS OF MEASUREMENT

Measuring visual acuity in animals can be complicated. Unlike with humans, one cannot simply ask an animal to read letters off of a chart. In order to determine if an animal is able to resolve an image, there needs to be some response to the image with which experimenters can use to gauge the visual acuity of the animal. As described by Cameron et al. (2013), there are two reflex responses that are commonly used to ascertain the visual acuity of an organism. The first of these is the optokinetic response. An optokinetic response is where a restrained animal moves its eyes in order to track a moving pattern. An animal is inclined to track the stripes in order to stabilise the image on the retina. These eye movements can be precisely recorded and used to determine if the fish is able to perceive the stripes passing in front of it. By monitoring the response in eye movement while changing the stripe widths, the visual acuity of the fish can be determined.

The second is the optomotor response. An optomotor response is when a fish swims in the same direction as a moving pattern of stripes. If the fish can resolve the stripes, it swims with their direction of movement in order to stabilise the image on its retina (Robinson et al. 2011). If the fish cannot resolve the stripes however, the retina will not detect movement, and the fish will not follow the stripes. Using this information, a fish can be presented with an array of different stripe widths in order to hone in on the smallest stripe size that it can resolve, and from there, calculate the fish's visual acuity.

There are both pros and cons for each of these approaches to measuring visual acuity. Determining if an optomotor response is occurring is considered more subjective than doing the same for an optokinetic response. This is because optomotor responses are often recorded by a human observer, whereas the rapid and subtle nature of an optokinetic response means that computer analysis is normally involved to make judgement on whether a response is occurring or not (Cameron et al. 2013). Despite this however, an optomotor response has still proved to be a useful tool in determining visual acuity (Harden Jones, 1963; Herbert et al. 2002; Robinson et al. 2011; Robinson et al. 2013) and in diagnosing visual impairment (Cameron et al. 2013). The optokinetic response,

although it represents a more precise and objective determination of what a fish can see, also requires more equipment, software is all but essential in order to perform eye tracking, and importantly, requires the subject to be restrained. This aspect of optokinetic work is sub-optimal in regards to this study, as yellow-eyed mullet are a surprisingly delicate fish, and unnecessary handling of the fish should be avoided. A benefit of utilising an optokinetic response is that it allows you to examine the visual acuity of organisms which may not be equipped to deal with the physical rigors of a motor response, such as fish larvae (Cameron et al. 2013). However, this was not a consideration given that this body of work deals with adult yellow eyed mullet.

Given the considerations just covered, the use of the yellow-eyed mullet's optomotor response was determined to be the best metric to use to determine the visual acuity of the species.

The experiments described in this chapter aim to determine the effect that increasing water turbidity has on the visual acuity of yellow eyed mullet. Put in other words, "are mullet adapted to be able to rely on their sense of sight even in the turbid conditions so often found within their home ranges?" The methodology below follows the example set by Herbert and Wells (2002), Herbert et al. (2003), and more recently Robinson et al. (2011) and Robinson et al. (2013).

3.2 MATERIALS AND METHODS

Experimental setup:

Twelve mullet were used for these experiments (average weight: 73.8 g, average length: 197.2 mm), and 9 for controls (average weight: 72.1 g, average length: 196.8 mm). In all cases, fish were tested individually. As depicted in figure 3, optomotor experiments were performed in a round clear perspex tank (d), 1m in diameter, the base lined with opaque white perspex, and filled with 40 L of seawater. Concentrically surrounding the tank was a polystyrene ring lined with uniform alternating black and white stripes (c). This polystyrene ring sat upon a wooden turntable which could be rotated clockwise or anticlockwise at 4 RPM by an electric motor (g). To provide a uniform light source, a cylindrical lighting tube was fixed in the centre of the tank (e). Having the light positioned in this manner meant there were no reflections on the surface of the water (for better filming) and ensured the fish were not provided with positional cues. In order to further ensure that the fish were unable to orientate themselves to features outside of the optomotor apparatus, the optomotor experiments were conducted within a tent (b) constructed of blackout curtain lining. At the apex of the tent, a small opening allowed a Canon video camera (a) (Legria HF20) to protrude through and film the experiments from above. All experiments were conducted within a windowless, temperature controlled room at 15 degrees Celsius, with the light (e), as the solitary light source.

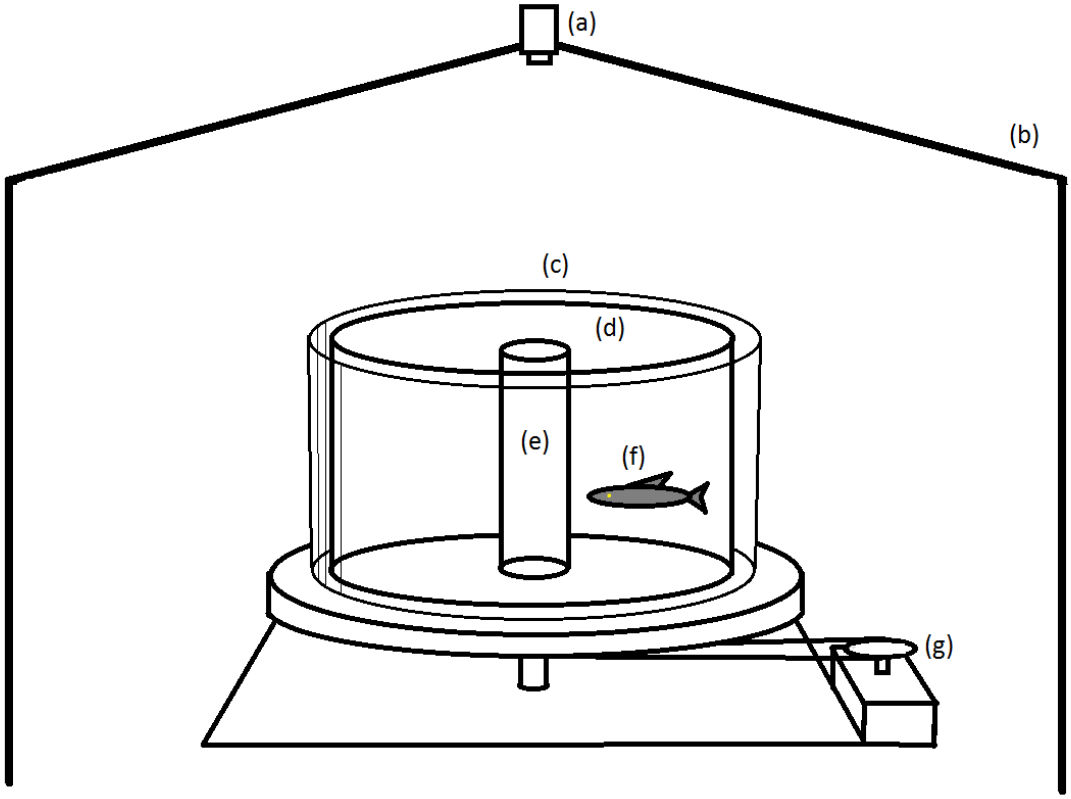

Figure 3 "Optomotor Setup": (a) Video camera, (b) tent, (c) polystyrene ring, (d) optomotor tank, (e) light tube, (f) a mullet, (g) electric motor.

General experimental procedure:

Each mullet was taken from the home tank and placed in the optomotor tank the afternoon before experimentation (where the light dark cycle was maintained until experimentation began), allowing them to acclimate to the optomotor apparatus overnight. Experimentation began 17 hours later the following morning with the introduction of the polystyrene ring being fitted around the tank. The day's experimentation would proceed with optomotor experiments being conducted over a range of turbidity levels (10, 50, 90, 130, 170 NTU). Preliminary testing had indicated that 2.5mm wide stripes (acuity angle: 1, minutes of arc: 60) invoked the strongest optomotor response in the yellow-eyed mullet, so this was the chosen stripe set to be used in conjunction with varying turbidity. All trials ran for 3.5 minutes (including a 30 second unrecorded period at the beginning of each experiment to mitigate the effects of any startle response). A number of control experiments were filmed using the same procedural framework. Broadly speaking, these controls fell into two groups; rotating controls, where the fish were exposed to a uniform grey rotating polystyrene ring in place of the striped one,

and static controls where fish were exposed to both striped and grey polystyrene rings that remained stationary for the duration of those trials. All experiments were filmed, and later reviewed for data collection.

The optomotor response to the movement of the rings was quantified by determining directional bias using the methods of, Herbert and Wells, 2002; Herbert et al. 2002; Herbert et al. 2003; Robinson et al. 2011, 2013. The direction of the fish's movement (clockwise, anticlockwise, no response) was recorded every 30 seconds (starting at t = 30 sec). The number of instances of the fish moving in the direction of stripe rotation (D^+), the number of instances of the fish swimming in the opposite direction of the stripe rotation (D^-), and the number of instances of the fish being non-directional or stationary (D) were all recorded. Directional bias was determined using the formula:

$$x = 100\left(\frac{D^+ - D^-}{D^+ + D^- + D}\right)$$

A directional bias of greater than 0.25 indicates that a positive optomotor response is occurring (Harden Jones 1963; Herbert and Wells 2002; Herbert et al. 2002, 2003).

Static controls:

Before beginning the acuity experiment, some static controls were necessary in order to show that any directional biases shown by the fish during the acuity experiments were not caused by the sound/vibrations of the electric motor, or by the mere presence of black and white stripes (regardless of their movement). These controls would also determine whether or not mullet show laterality in their swimming behaviour. To this end, trials were conducted with stationary polystyrene rings. This was achieved by disengaging the drivetrain of the electric motor so that it would still produce all the same sound and vibration, but not rotate the turntable upon which the polystyrene ring sat. This style of control was conducted using both the striped ring and the grey control ring. The striped ring stationary control was conducted in tandem with the acuity experiments. The grey ring stationary controls were conducted both before and after the acuity experiments. All control were repeated at 10, 50, 90, 130 and 170 NTU.

Effect of turbidity on visual acuity:

To perform the acuity experiments, the striped polystyrene ring was fitted around the optomotor tank. One then the other, in a random order, trials were conducted with the polystyrene ring rotating clockwise and anticlockwise. Each fish underwent this procedure at 10, 50, 90, 130 and 170 NTU again in a random order.

Before and after every acuity trial, a rotating control was performed. This was to help show that any directional bias that the fish displayed during its exposure to rotating stripes was in fact an optomotor response, and not a swimming response triggered by other factors. To achieve this, each fish undertook both clockwise and anticlockwise rotating trials (in a random order) of the same procedure as before, but with a uniform grey polystyrene ring in place of the striped one.

For statistical analysis, stationary controls (both striped and grey) underwent regression analysis in order to see if any given response correlated with turbidity and check for laterality. For the optomotor experiment and accompanying rotating grey control, the data was plotted as a range-standard-deviation box plot (figure 8), and relationships that appeared to be worth investigating (i.e. relationships with even a remote chance of significance) were examined using t-tests. T-tests were selected on the basis on which set of assumptions were met by the data.

3.3 RESULTS

3.31 Static controls:

When presented with a stationary striped ring, mullet show no preferrence for swimming in either direction at any turbidity, instead preferring to not move at all. A regresssion analysis was performed for each response type across the range of turbidities and the p-values and R-squared values of “no response” (p-value: 0.84, R-squared: -0.02227), “clockwise response” (p-value: 0.9125, R-squared: -0.02297), and “anticlockwise response” (p-value: 0.8538, R-squared: -0.02244) all indicate that none of the response types have a significant relationship with turbidity.

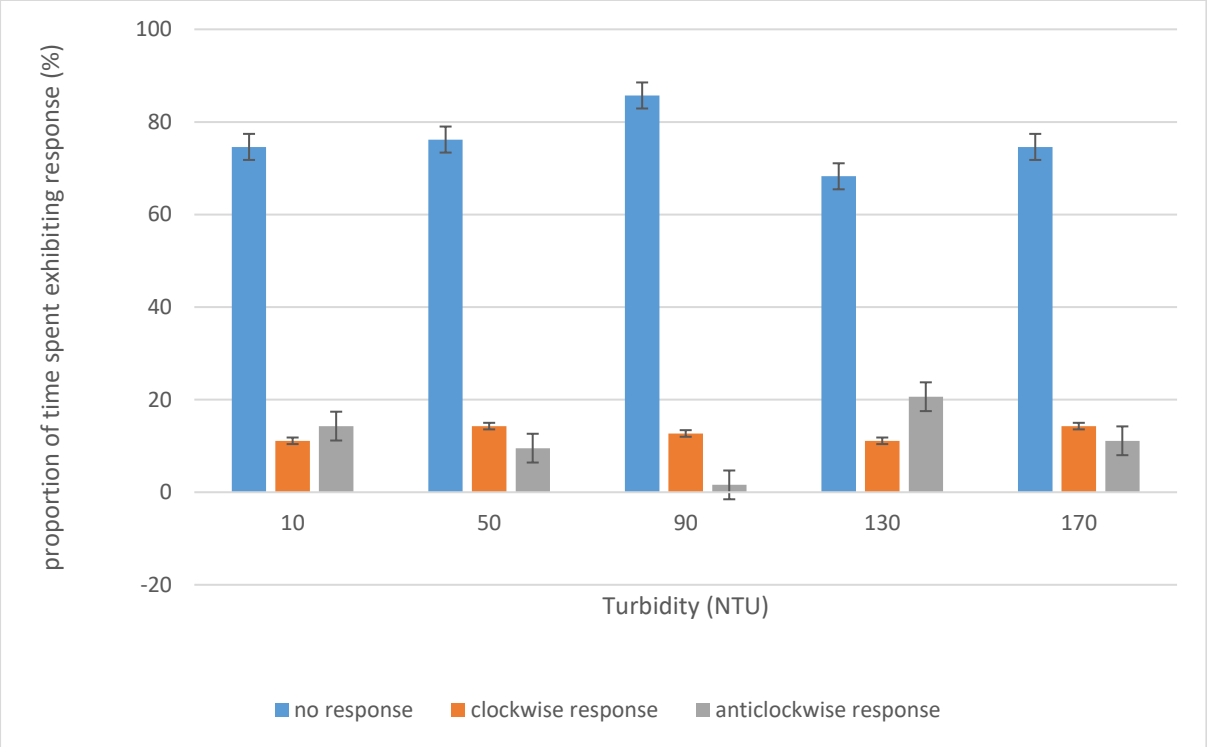

Figure 4 Swimming response elicited by stationary stripes.

When presented with a stationary grey ring before the acuity experiements, mullet show no preferrence for swimming in either direction at any turbidity, instead preferring to not move at all. Regression analysises were performed of each response type across the range of turbidities and the p-values and r-squared values of “no response” (p-value: 0.1791, R-squared: 0.01929), “clockwise response” (p-value: 0.2691, R-squared: 0.00573), and “anticlockwise response” (p-value: 0.3547, R-squared: -0.002844) indicate that none of these responses have any kind of significant relationship with turbidity.

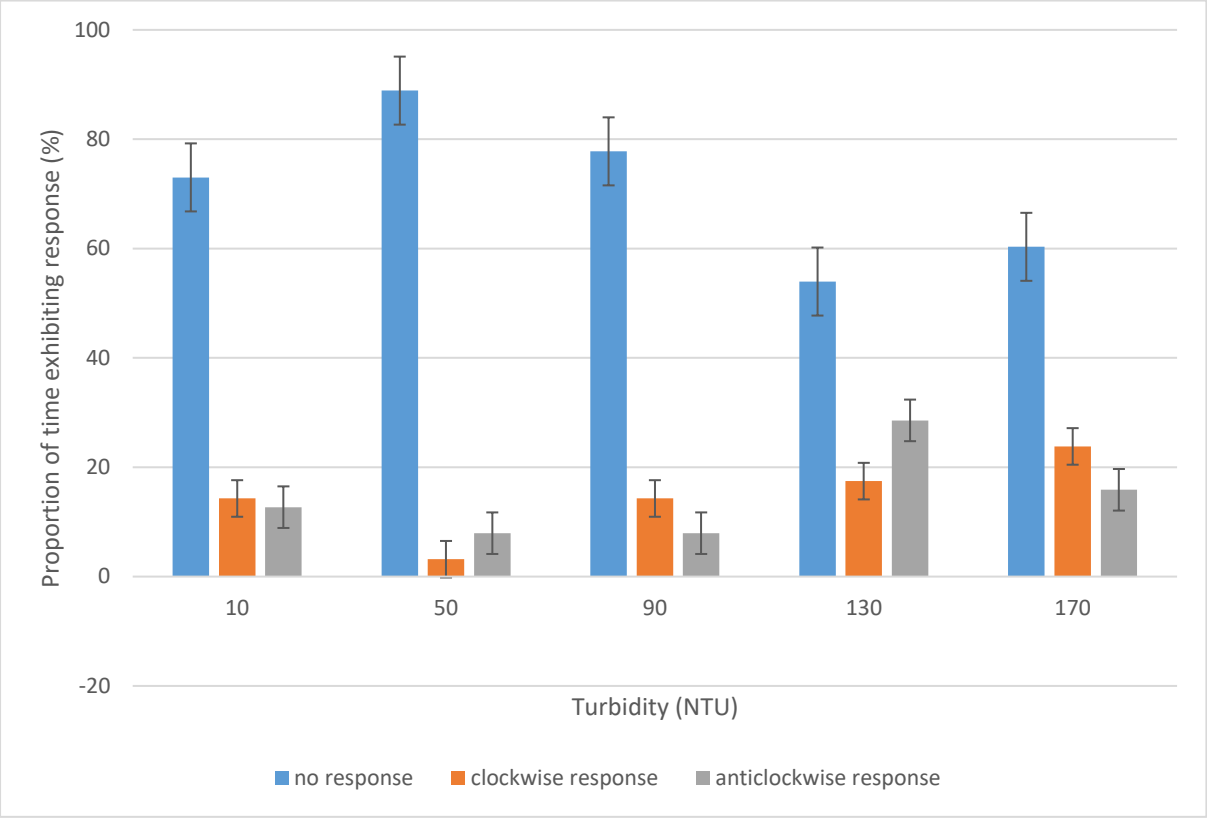

Figure 5 Swim response elicited by stationary grey control ring (before rotating striped trials).

When presented with a stationary grey ring after the acuity experiements, mullet show no preferrence for swimming in either direction at any turbidity, instead preferring to not move at all. Regression analysises were performed on each response across the turbidity levels and the p-values and R-squared values of “no response” (p-value: 0.8646, R-squared: -0.02256), “clockwise response” (p-value: 0.9051, R-squared: -0.02291), and “anticlockwise response” (p-value: 0.7159, R-squared: -0.02007) all indicate that there is no significant relationship between turbidity and any of the repsonses.

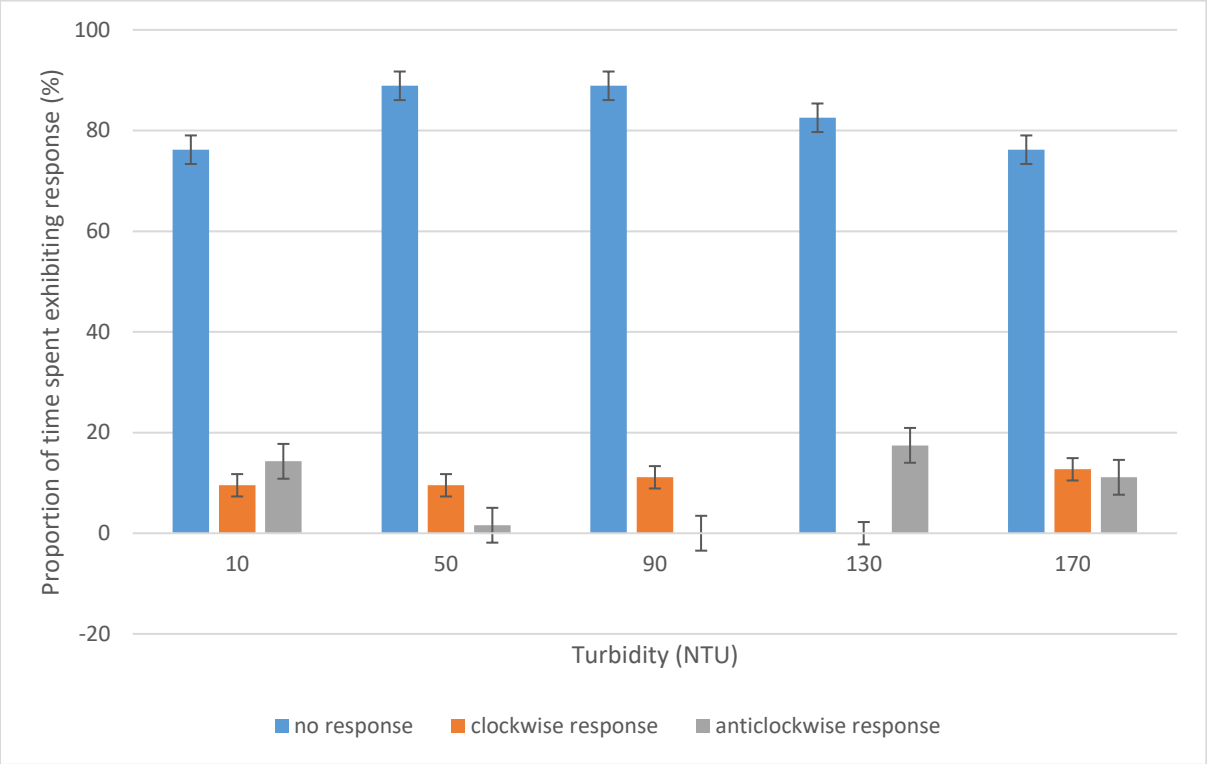

Figure 6 Swim response elicited by stationary grey control ring (after rotating striped trials).

3.32 Optomotor and rotating controls:

A directional bias of over 25% is considered indicative of an optomotor response (Harden Jones 1963; Herbert et al. 2003, 2002; Herbert and Wells 2002). The rotating stripes at 10 NTU produced a directional bias result of 25.3%, indicating a weak, but significant optomotor response that was statistically different from its control (p-value = 0.02285) and from all other turbidities in which no positive optomotor response was evident (figure 7a, b). Likewise, the controls did not, at any turbidity, produce a response that could be considered to be an optomotor response. The control trials using the rotating grey ring were performed both before and after the trials using the striped ring, however they produced such uniform results that only the "after" set is displayed here.

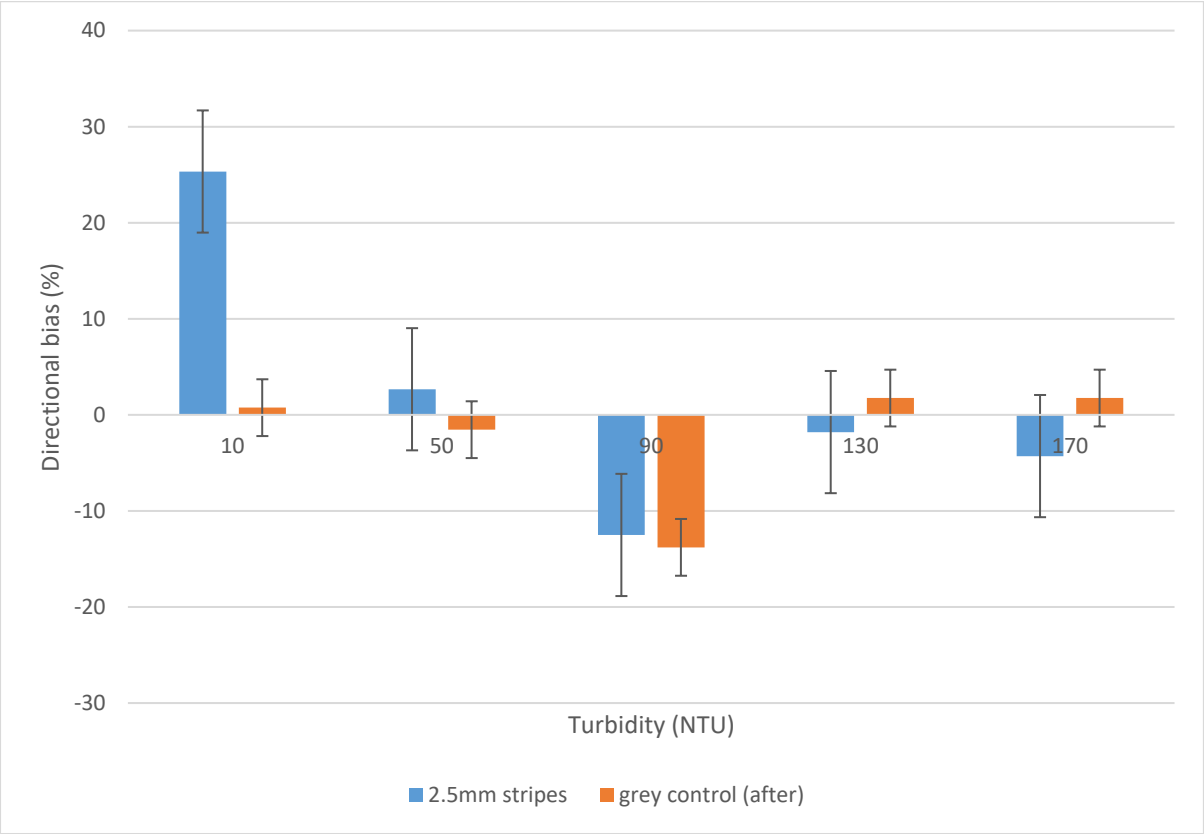

Figure 7 directional bias of mullet at a range of turbidities. Results are mean ±SEM.

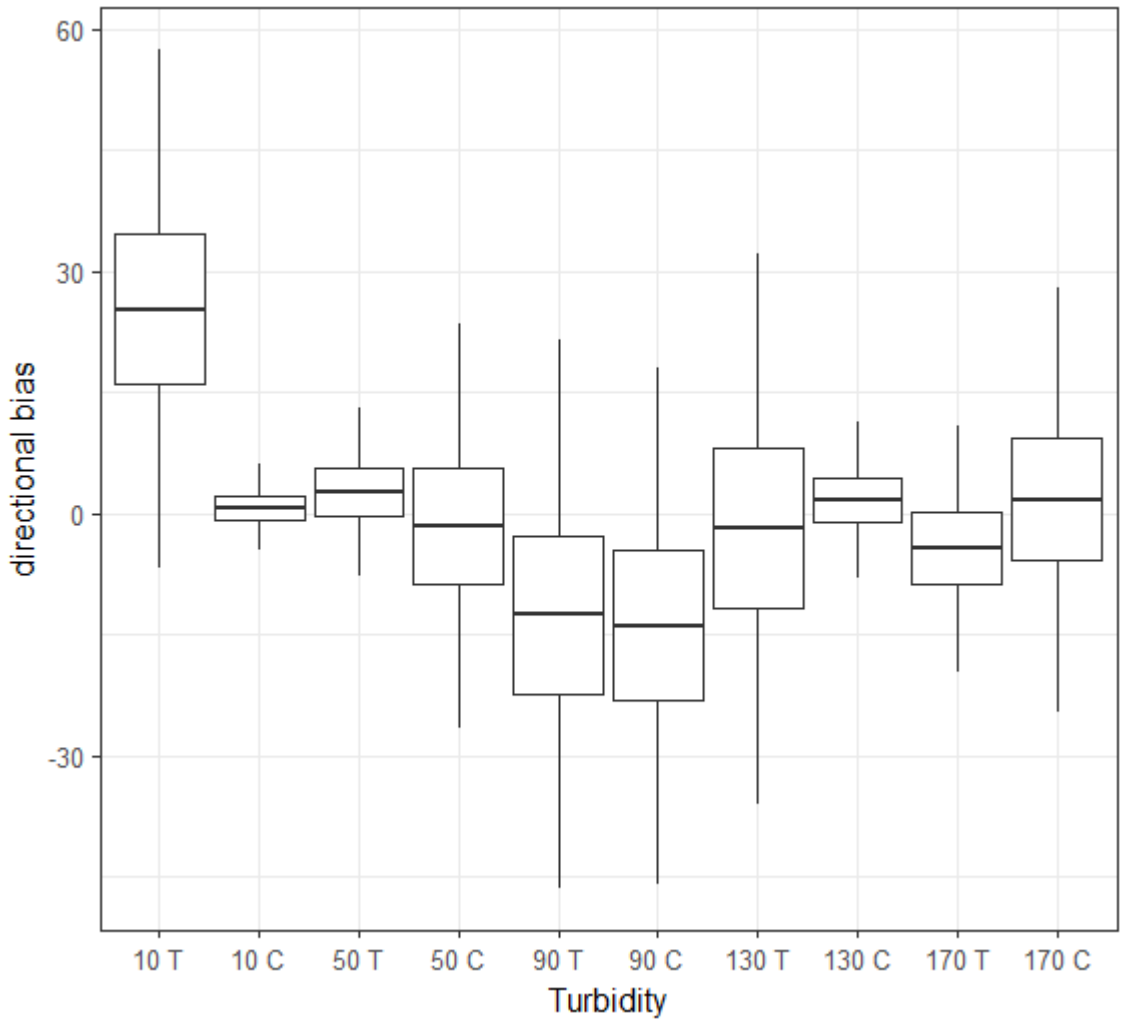

Figure 8 Range-standard-deviation-mean box plot of rotating striped (T) and rotating grey control (C).

3.4 DISCUSSION

Yellow eyed mullet display an optomotor response at 10 NTU, but not at any of the other turbidity levels at which they were tested. The swimming response of the mullet to rotating 2.5mm wide black and white stripes showed a directional bias above 25% (25.3%). This level of bias is considered to constitute an optomotor response (Harden Jones 1963)(Herbert et al. 2002)(Robinson et al. 2011, 2013), and is significantly different to the rotating grey control (p-value = 0.02285). At no other turbidity does the directional bias come close to 25%, and no other responses show a significant difference to their controls.

Mullet showed no directional preference for swimming direction, at any turbidity, when presented with a stationary uniform grey control ring (figures 5 and 6) or a stationary striped ring (figure 4), and instead they predominantly remained motionless throughout the trials. The absence of a directional bias indicated that the mullet had no inherent inclination to swim in either direction when placed in the experimental apparatus, and that the stripes needed to be in motion to produce a positive response. Therefore any directional bias shown in later trials could be more confidently attributed to being an optomotor response. Checking for an innate directional bias was crucial, as many fish exhibit laterality, or handedness, while swimming. Simply put, laterality is an assymetry of brain function where one body part is used preferentially over its symetrical counterpart (Bisazza et al. 1997). In the case of fish, this is commonly recorded as the preferential use of one eye, which influences swimming behaviour as fish preferentially turn in one direction in order to keep the dominant eye focussed on any given stimulus. Bisazza et al. (1997) tested 5 species of poeciliid fish, and found, when presented with an obstacle with potential mates on the far side, 3 species preferrentially navigated the obstacle to the left, and 2 to the right, indicating which eye was fixated on the potential mates, and therefore dominant. Laterality, in some form, is found in many fish, birds and mammals, and is very important to check for first before commencing optomotor experiments.

It is clear from the data that the optomotor response diminished entirely somewhere between 10 and 50 NTU, and that further increases in turbidity had no return in further changing the response. This is similar to the result found in Benfield and Minello (1996) where the distance at which humans could detect a stationary object through water reduced quickly as particulate matter was mixed into the water, increasing the turbidity. However, the effect of further adding particulate matter, after the initial decrease, produced diminishing returns, with the greatest decline of reactive distance occurring as turbidity increased from low to moderate levels. The response optomotor response

measured from the mullet makes sense in light of the turbidity data gathered from the Avon-Heathcote Estuary; referring to figures 1 and 2 it can be seen that turbidity in the estuary is typically under 50 NTU. This is similar to results found in an investigation performed at Ohio State University. Robbins and Gray (2016) tested the optomotor response of the cichlid *Pseudocrenilabrus multicolour victoriae* to 35mm wide black and white stripes in different levels of turbidity. This is a species of fish that, like the yellow eyed mullet, live in areas prone to high turbidity levels. It was found that male specimens ceased exhibiting an optomotor response at 62 NTU, while females did the same at 53 NTU. While the yellow-eyed mullet in the present work ceased exhibiting an optomotor response at some turbidity between 10 and 50 NTU, it is possible that the visual acuity of the two species are comparable, as the cichlids were tested with a considerable thicker stripe width.

Preliminary testing to see what width of stripes would evoke the strongest optomotor response in the mullet indicated that while 2.5mm stripes were the best option, no stripe width produced a strong optomotor response. This is unusual, as fish like mullet, which have large eyes and school strongly, would be expected to exhibit a strong optomotor response. Preliminary testing also indicated that mullet did not exhibit a stronger response when placed inside the optomotor apparatus in groups of three. If mullet are in fact inclined to exhibit a stronger optomotor response than that seen presently, it remains unclear why this was not the case with this particular experimental set up.

Beyond Robbins and Gray (2016) and the work presented currently, research pertaining to the effect of turbidity on the optomotor response is very limited. However, there is considerably more work which indirectly describes effects of turbidity on fish vision by measuring changes in visually mediated behaviour. Some of the work already mentioned in section 3.1 describes how elevated turbidity impacts visually orientated fish by reducing their ability to find and catch items (Benfield and Minello, 1996; Rowe and Dean, 1998; De Robertis et al. 2003; Lowe et al. 2015), their ability to learn and identify predators (Ferrari et al. 2010), and by making them adopt more cautious behaviours due to an reduced ability to detect predators (Leahy et al. 2011).

Suspended materials in water disrupt the vision of aquatic organisms because of the way they interact with light. Unimpeded by solids, visible light will penetrate water up to 1 km deep depending on the wavelength of that light. In the case of turbid waters however, which are clouded by huge numbers of tiny suspended particles, the passage of this light is interfered with, as the particles reflect light back, or refract it, altering the direction in which the light is travelling. It is this scattering of light that gives turbidity its characteristic “cloudiness”. By disrupting and distorting images before they even reach the eye, turbidity has a large effect on the vision of fish. However, it

is of note that different wavelengths of light are effected differently by the presence of suspended solids in the water. Robinson et al. (2011) found that juvenile snapper, *Pagrus auratus*, rely more heavily on longer wavelengths of light, which are more prevalent in the turbid estuarine areas in which they tend to live. More developed snapper on the other hand, are more sensitive to longer wavelengths which are characteristic of the clear open ocean environments snapper move to when they are mature enough. This means that the effect that any given level of turbidity has on the vision on any given species of fish will depend on the wavelengths of light that the species, or in some cases even the age, of fish is adapted to perceive. Also of consideration is that turbidity is hypothesised to, in some specific cases, improve the contrast between a fishes prey items and the background, which improves reaction distance (Utne-Palm, 2004). In short this is because turbidity filters out objects at distance, meaning that anything at closer range is stands out on a featureless background.

Lunt and Smee (2015) explains how chemosensory-reliant predators are not effected by turbidity, and because of this, an increase in environmental turbidity tends to increase the abundance of these species, as more visually dependent species are less able to compete for resources. Some fish can utilise both visual and chemical information to find food. A study on a number of native New Zealand juvenile freshwater fish (inanga – *Galaxias maculatus*, banded kokopu – *Galaxias fasciatus*, koaro – *Galaxias brevipinnis*, common bullies – *Gobiomorphus cotidianus*, red finned bullies – *Gobiomorphus huttonil,* smelt – *Retropinna retropinna*) found that turbidity had differing effects on their feeding rates on *Daphnia* (Rowe and Dean, 1998). The feeding rates of the banded kokopu, smelt, inanga and common bullies were reduced by turbidity, while red finned bullies and koaro were comparatively unaffected. The differences between species is explained by their differing sensory dependencies, with those most heavily reliant on vision being the worst affected. Some of these fish may have been able to better utilise tactile and chemical signals to locate prey, allowing them to compensate, at least partially, for a lack of vision in turbid environments. Some fish can even adapt to turbid environments at a genetic level. Ehlman et al. (2015) compared the gene expression of guppies, *Poecilia reticulate*, that had been reared in clear water versus those that had been reared in clear water. They found that turbid-reared guppies had a change in the opsins they produced, shifting their vision from mid-wave sensitive to long-wave sensitive, allowing better vision in turbid conditions, much like the developmental differences seen in snapper in Robinson et al. (2011).

In summary, from the optomotor work conducted it is clear that under broad spectrum white light, yellow eyed mullet do have reduced visual acuity under turbid conditions, losing all optomotor response to 2.5mm black and white stripes somewhere between 10 and 50 NTU. The effects that impaired vision could have upon the swimming ability of yellow eyed (and feeding by proxy), their

ability to school, and their ability react to predators are dealt with in chapter 4. From the optomotor experiments presented in this chapter it is impossible to say if mullet eyes are more sensitive to certain wavelengths of light, how well equipped they are to compensate for a lack of vision with other senses, or if they are able to display a degree of developmental plasticity. The visual acuity of the yellow eyed mullet should be more closely examined in the 10 to 50 NTU range to determine the precise relationship between visual acuity and turbidity. As the optomotor experiments produced a weaker response in the fish than anticipated at 10 NTU, future work using the optokinetic response may be able to either confirm what was found here, or bring new information to light, and could be done so with different parts of the electromagnetic spectrum. Feeding experiments with varied turbidity are another avenue for future enquiry, as are rearing experiments to examine the possibility of genetic plasticity. These will give indication if the mullet are able to compensate for the effects of turbidity on their visual acuity, which would be crucial for their ability to thrive in the ever changing waters of the Avon-Heathcote estuary, and other visually variable habitats throughout New Zealand.

4 EFFECT OF TURBIDITY ON THE SWIMMING MOVEMENTS OF YELLOW-EYED MULLET (*ALDRICHETTA FORSTERI*)

4.1 INTRODUCTION

4.11 Spontaneous movement and grouping behaviour

Activity and turbidity:

Many fish species are constant and active swimmers. Being on the move can bestow a number of advantages to fish, including passive ventilation of the gills and a higher frequency of mate and prey encounters. Turbidity, however, can interfere with these advantages and nullify the benefits of the behaviour. To this end, it is unsurprising that many species of fish are known to reduce activity levels in increased turbidity (Leahy et al. 2011) in order to conserve energy in a situation that would otherwise mean lower feeding rates and thus less energy available for growth and reproduction. Leahy et al. (2011) investigated the behaviour of the spiny damselfish, *Acanthochromis polyacanthus*, finding that the damselfish decreased activity by 23 percent at a turbidity of 24 NTU.

In accordance with this, a study on the effects of turbidity on Atlantic cod found that increased turbidity lowered the activity levels of cod (Meager and Batty, 2007). The cod were placed in a tank of a particular turbidity and their movement was monitored in the presence and absence of olfactory prey cues. At intermediate and higher levels of turbidity, the activity levels of the cod were significantly reduced. The explanation proposed for this is that in turbid water a fish has less prey encounters, and so to conserve energy swims slower during times when there is no prey present. This is backed up by the fact that when exposed to a prey odour cue, the activity levels of the cod slightly increased, showing that higher activity is only a viable option when food is an imminent prospect.

Schooling behaviour and turbidity:

Schooling is an important behaviour for fish. More sets of eyes allow the group to spot predators and prey faster, school numbers and movement can confuse predators, and individuals do not have to look far for a mate (Pitcher, 1986). Generally, vision is the primary sense involved in the schooling of fish, although not the only one. Olfactory cues as well as tactile ones (detected by the lateral line) are also used to facilitate schooling behaviour, although the reliance on each of the three varies from species to species. Sensory isolation experiments have managed to demonstrate the importance of the various systems. Pitcher et al. (1976) described how saithe, *Pollachius virens*, with

eye covers were able to school along with a group of unblinded individuals, but not when the blinded fish also had their lateral lines severed from the nervous system. Fish have also been shown to, when deprived of vision, prefer areas that smell like conspecifics; a mechanism thought to help get schools in the same area overnight (Keenleyside, 1955).

Hemmings (1966) describes how two schooling fish in a tank separated by a plastic sheet will sit very close together, but once the sheet is removed, they will separate to the normal spacing between schooling individuals. There is a 'push-pull' type interaction occurring here, where fish are visually drawn to each other, but when they get too close, tactile information tells them to separate, thus maintaining a balance at the ideal spacing for schooling. Hemmings (1966) goes onto describe how the thinking that fish schools break apart at night due to lack of visual information is an oversimplification, and that chemical cues take over allowing fish to maintain a looser 'aggregation' in lieu of a directional school. Gray et al. (2014) conducted a study on the effects of turbidity on a group of imperilled shiner fish species (*Notropis heterolepis, Notropis heterodon, Notropis anogenus, Notropis bifrenatus, and Notropis volucellus*) in North America. The species of shiner which are most accustomed to turbid environments were largely unaffected by the experimental low levels of turbidity. The species native to clearer waters experienced decreased schooling behaviour and, in a conservation context, are therefore considered to be at greater risk from increasing turbidity in waterways.

4.12 Response to a simulated predator

Some species actually benefit from elevated levels of turbidity. Ohata et al. (2011) exposed ayu, *Plecoglossus altivelis*, and red sea bream larvae, *Pagrus major*, to predation over a range of turbidities. The predators (separately tested) were jack mackerel juveniles, *Trachurus japonicus*, (a visual predator) and moon jellyfish, *Aurelia aurita*, (a tactile predator). The ayu and bream larvae enjoyed far higher survival rates under turbid conditions when exposed to the mackerel, while turbidity had a far lesser effect on predation by the jellyfish. This allows us to conclude that in some instances, water turbidity may benefit some species by hiding them from visual predators. In a follow up study, Ohata et al. (2014) observed the schooling behaviours of ayu, Japanese anchovy larvae, *Engraulis japonicus*, and yellowtail juveniles, *Seriola quinqueradiata*, under different turbidites. It was found that ayu and Japanese anchovy larvae actually demonstrated stronger schooling behaviours under moderately turbid conditions than they did when the turbidity was zero. This manifested as a decrease in nearest neighbour distance and a decrease in separation angle (i.e. fish were more parallel). These results make sense in the light of the life histories of these species –

both of which often live in turbid coastal waters. The yellowtail, an open water fish, did not fare so well, with any amount of turbidity having a detrimental effect on its schooling abilities.

Bruton (1985) states that turbidity reduces a fishes risk of aerial predation by obfuscating its position. However Grémillet et al. (2012) demonstrates that this is not necessarily always the case, showing that turbidity does not protect the fish found in Macedonian lake systems from predation by *Phalacrocorax carbo*, the great cormorant. Cormorants and related birds, such as shags, are commonplace in estuaries and coastal areas around the world, and given that they are commonly found near coastal waters and estuaries (two environments which are both often characterised by variable turbidity) it is quite possible that their hunting is generally not impaired by turbidity.

The literature has many examples of fish that deal well with turbidity, as well as those which do not. As previously mentioned, yellow-eyed mullet are known to live in a variety of habitats, fresh, marine and brackish, so it is difficult to predict how they cope with elevated levels of turbidity in the Avon-Heathcote estuary just by using existing literature on other species. The research in this chamber aims to assess how turbidity effects the swimming activity, the grouping behaviour, and the avian predator response of the yellow eyed mullet. Of course, all of these behaviours may be unchanged by turbidity which would indicate that turbidity has little to no effect on the mullet, and that the mullet are adapted to be able to maintain normal behaviours at a large range of turbidities. An increase in any of these behaviours correlating with an increase in turbidity would possibly be indicative that mullet are best adapted to live in turbid waters, though conversely tighter grouping and an increased response to a simulated avian predator could mean any number of things, including that the mullet perceive themselves to be at greater risk. Lastly, if activity, grouping behaviour, and predator response are inversely correlated with turbidity, that would most likely suggest that mullet are negatively affected by increasing turbidity. Given that the yellow eyed mullet occupy an estuary which exhibits extremely variable turbidity (figures 1 and 2), the results of the present investigation will give good insight on how well suited mullet are to this habitat.

4.2 MATERIALS AND METHODS

Experimental setup:

Activity and schooling experiments were performed in a round plastic tank, 1.5m in diameter, the inside of which was painted white in order to contrast with the fish. During experimentation, the tank was filled with 100L of seawater which was maintained at 15˚C. This tank sat inside a tent constructed of blackout curtain lining, blocking any visual features of the room that could have interfered with the experiment. At the apex of this tent, a small opening allowed a video camera (Canon Legria HF20) to film the experiments from above. Placed on opposite sides of the tank, two lamps provided the only light source during the experiments. These lamps were connected to a dimmer switch outside the tent, so their intensity could be adjusted without disrupting the experiment. The tank was divided into 5 equal sections in order to aid analysis. Five submersible water pumps, one in each section, were placed on the bottom of the tank, evenly arranged around the perimeter. These pumps pointed towards the centre of the base of the tank, in order to agitate the water and sweep settled porcelain dust off the bottom and back into suspension without creating a circular current around the tank. Experiments were conducted with 4 groups of 4 fish. Each group of 4 fish was added to the tank the afternoon before experimentation (where the light dark cycle of the home tank was maintained until experimentation began), allowing them to acclimate to the setup overnight. Experimentation began 17 hours later the following morning.

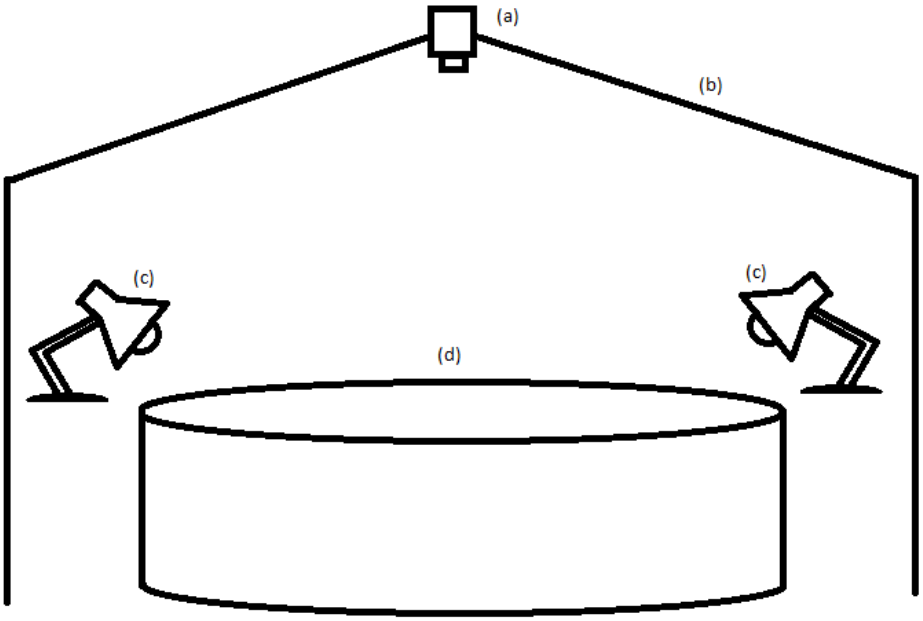

Figure 9 the behaviour setup in profile: (a) camera, (b) blackout tent, (c) dimmer lamps, (d) experiment tank.

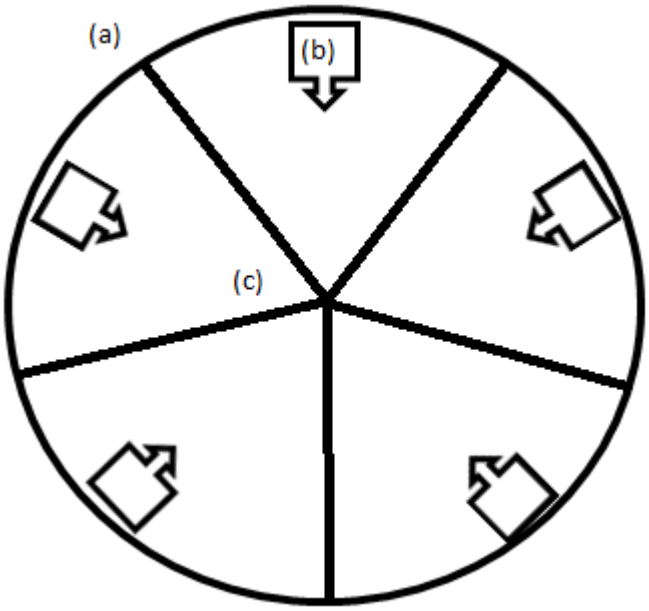

Figure 10 the behaviour setup from above (a) experiment tank, (b) pump r, (c) lines that indicate the zones of the tank

Experimental Procedure:

4.21 Spontaneous movement and grouping behaviour

In order to ascertain how different turbidity levels effected the activity levels of the fish, and how these turbidities effected the way the mullet would position themselves relative to one another, a simple series of experiments were conducted. In these experiments, as described above, fish were tested in groups of 4 (average weight: 97g, average length: 212mm). These fish were allowed to swim freely within the experimental setup for 5 minutes while the camera filmed from overhead. Each group repeated this procedure at each turbidity level (10, 50, 90, 130, and 170 NTU) in a randomly determined order. In order to quantify the activity of the fish in these trials, 3 minutes of the footage taken from each of the trials was examined. Each fish, in each group, was monitored. A fish was said to be occupying a particular zone of the tank if over half its body length was in that zone. A tally was taken of every time a fish changed what zone of the tank it was occupying over those 3 minutes. This way, an active fish would be represented in the data as a fish with a high tally of zone crossings. In order to quantify the grouping of the fish, the experimental footage was sampled at 30 second intervals. At each interval, a note was taken of how many fish occupied the

most occupied zone at that given time. In trials where fish tended to be closer together, we would expect to see higher recorded values for the largest number of fish in one zone.

4.22 response to a simulated predator

A similar experimental procedure was used to measure the response of the fish to a simulated predator. The fish, again in groups of 4, were allowed to swim with the experimental setup with filmed from overhead. They were left to swim normally for 5 minutes before, using the dimmer switch, the lights on either side of the tank were simultaneously dimmed and then brought back up to full brightness rapidly, simulating the shadow created by a seabird flying overhead. Filming continued for another 5 minutes, capturing any change in behaviour the fish displayed post-"shadow". These experiments were repeated at the full range of turbidities (10, 50, 70, 130, 170 NTU) in order to determine if the response to the "shadow" changed with turbidity. The response to the "shadow" was quantified in two ways. Firstly, as before, the movement of each fish between zones was tallied. This was done over a 30 second period before and after the "shadow" with the darkest point of the "shadow" being treated as the reference point.

For statistical analysis, the activity data was plotted as a range-standard-deviation box plot (figure 12), and relationships that appeared to be worth investigating (i.e. relationships with even a remote chance of significance) were examined using t-tests. The grouping data underwent an anova and a Tukey's multiple comparisons test. The predator response data, for each turbidity level, underwent an anova and then a Tukey's multiple comparisons test.

4.3 RESULTS

4.31 Spontaneous movement and grouping behaviour

Activity:

Activity is at its highest at 10 NTU at 41.75 zone changes, and is significantly greater than the activity at 50 NTU (p-value = 0.001325). From 50 to 170 NTU, activity is markedly lower, reaching no higher than 22.6 zone changes and getting as low as 9.6, and there is no significant difference between the activity levels recorded at these turbidities.

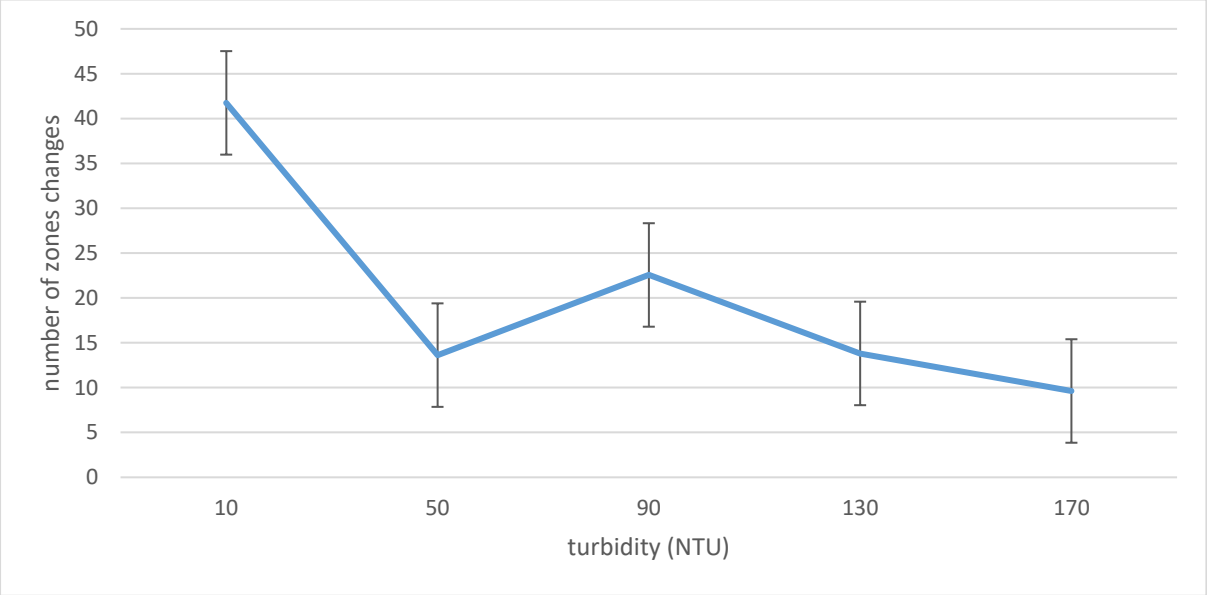

Figure 11 Average number of zones changes at a range of turbidities.

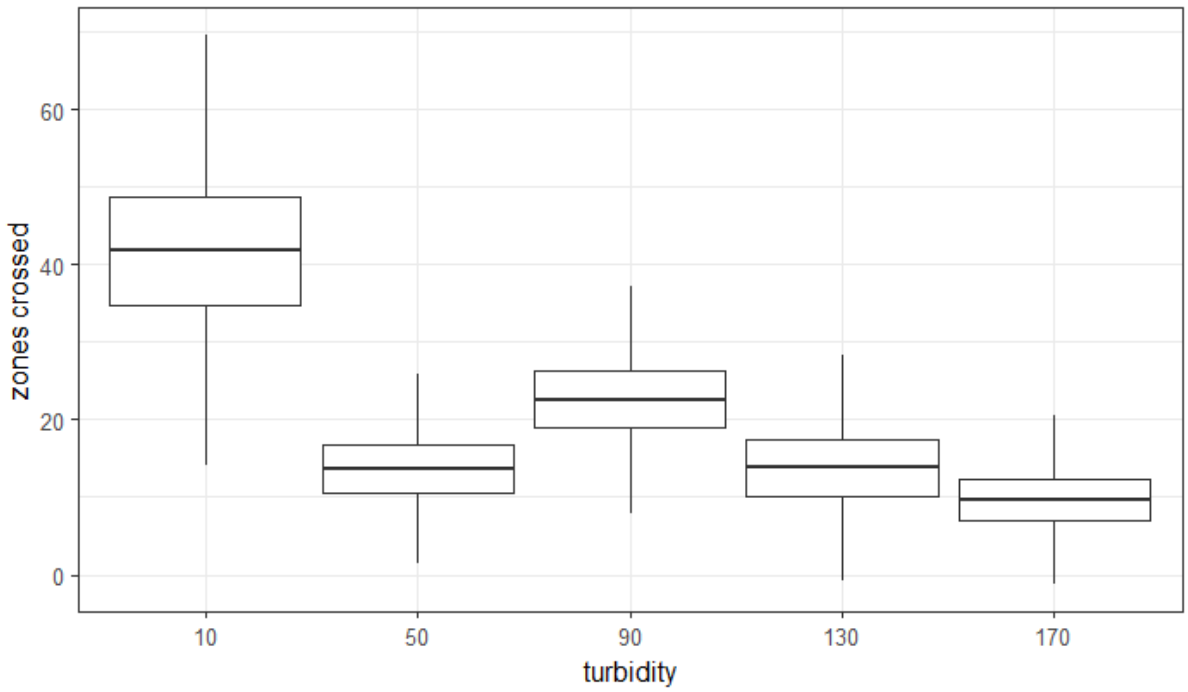

Figure 12 Range-standard-deviation-mean box plot of the average activity of mullet at a range of turbidities.

Grouping behaviour:

Only reaching as high as 2.3 and as low as 2, there is no significant difference between the grouping recorded at any of the turbidities as confirmed by Tukey's multiple comparisons test.

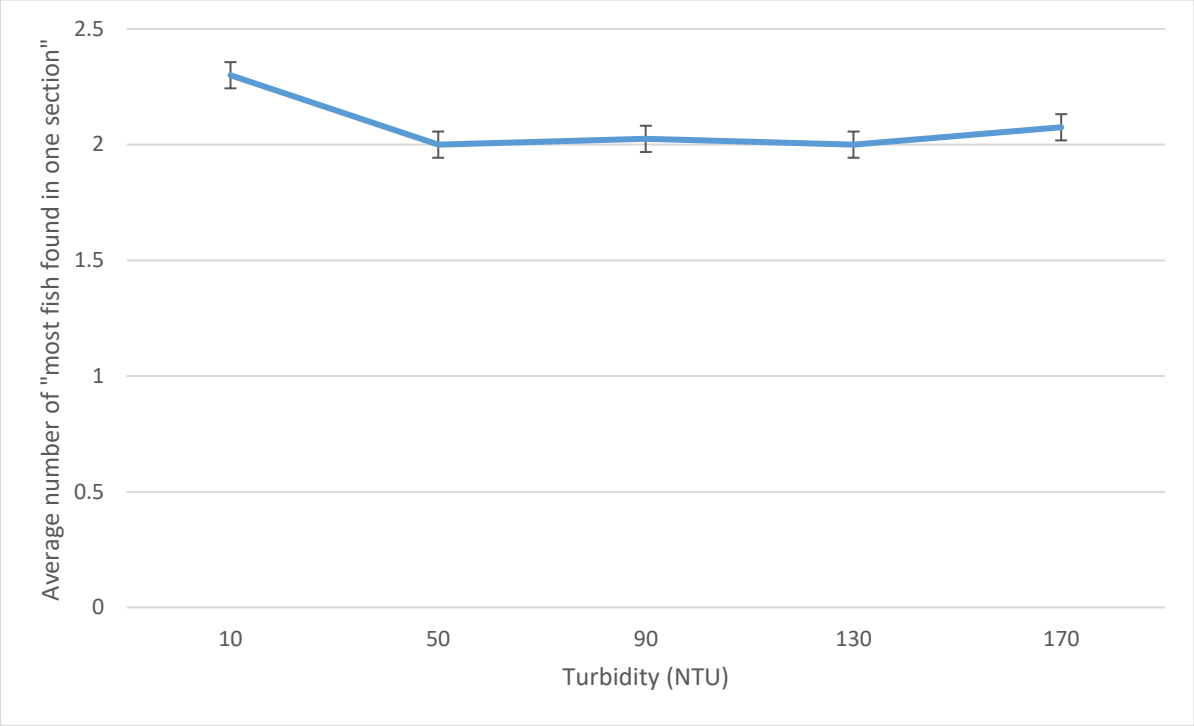

Figure 13 degree of grouping of mullet over a range of turbidities.

4.32 response to a simulated predator

At no turbidity is there a significant response to the passing shadow test, though 10 NTU comes close at “5 to 0 before” (p-value = 0.062).

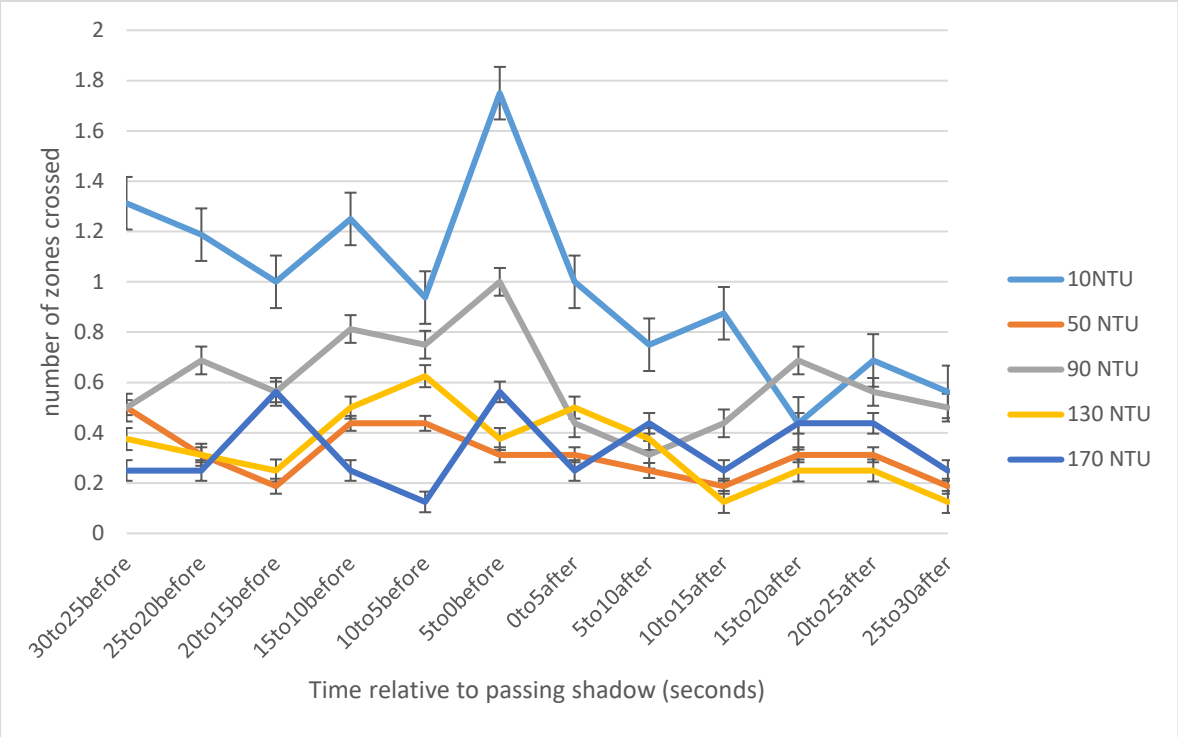

Figure 14 Time series of the number of zones crossed 30 seconds before and after a "passing shadow" event. Passing shadow event happens at time zero.

4.4 DISCUSSION

4.41 Activity and Grouping behaviour

The activity levels of the mullet were significantly higher at 10 NTU than at any other turbidity level (p-value = 0.001325). This suggests that, like many other fish, yellow eyed mullet do reduce their activity level at elevated turbidities. From looking at other examples in the literature, covered in section 4.1, we can conclude that this is likely because mullet are primarily visual foragers, and thus at higher turbidities, when their vision is impaired, it is not worth expending energy to look for food (Bruton, 1985; Meager and Batty, 2007; Leahy et al. 2011). If mullet activity exhibited no change with increasing turbidity, it would suggest that mullet are largely unimpaired by the turbidity levels found in the Avon-Heathcote estuary (figures 1 and 2), having well adapted vision, or relying on other senses for hunting. An increase in activity with increasing activity, though unusual, could indicate an attempt to compensate for reduced prey encounters as theorised by Granqvist and Mattila (2004).

There is no significant difference in the grouping behaviour of the mullet shown at any of the turbidity levels. Schooling and shoaling fish, such as mullet, use vision, tactile sense, and sometimes olfaction, in different combinations to facilitate these behaviours. Yellow eyed mullet, with their large distinctive eyes, were a likely candidate for a fish that is reliant on visual information to school properly. Although no relationship has been found, a change in grouping behaviour could have indicated a number of things. If grouping behaviour lessened with increasing turbidity, it could indication that the lack of visual information is allowing the “push” factor of the tactile sense to take over, separating the fish (Hemmings, 1966). On the other hand, tighter grouping with increasing turbidity would instead suggest that mullet are better adapted to turbid environments over clear ones, or perhaps that turbidity is creating stress in the fish, possibly from a perception of increased predation risk.

The lack of a response in grouping behaviour with varying turbidity perhaps indicates that mullet are generally reliant on tactile information to maintain distance to their neighbours, or at least their lateral line is sensitive enough to compensate for a lack of visual information at higher turbidites. As briefly discussed in section 4.1, Pitcher et al. (1976) demonstrated that blinded saithe, *Pollachius virens*, were able to school along with unblinded saithe, albeit with reduced performance. This ability to retain some schooling function without vision is attributed to the lateral line of the fish, as once this was severed, blinded fish lost all ability to school. Partridge and Pitcher (1980) expanded upon this work, performing further experiments with saithe which had been blinded or had their lateral lines severed. This paper determined that lateral lines are largely responsible for monitoring

the swimming speed and direction of neighbours, while vision is more important for the maintenance of relative position and angle between other fish in the school. This adds weight to the notion that mullet with reduced vision due to turbidity are still likely to be able to school due to sensory input of their lateral line, even though overall schooling ability would likely be reduced. Perhaps this reduction in schooling ability is not apparent in the present work because the experimental tank did not provide enough space to allow for a clear distinction in different grouping responses.

Regarding the effects of turbidity specifically on schooling, and already briefly described in section 4.1, Ohata et al. (2014) describes the schooling response of 3 species of fish to elevated turbidity. The finding of this paper indicated that some species have their ability to school enhanced by moderate levels of turbidity (ayu - *Plecoglossus altivelis* and Japanese anchovy -*Engraulis japonicas*), while others are impeded by any amount of turbidity (yellowtail - *Seriola quinqueradiata*). The reasons cited for the ayu and Japanese anchovy possessing a superior capacity to school in turbidity are that these species possess superior sensory organs. Ayu possess clusters of free neuromasts around their nose and eyes, providing a strong ability to detect tactile information from all directions. Japanese anchovy have well developed eyes that are sensitive to low levels of light, and have been suggested to utilise olfaction to keep schools close together when light levels are very low. While this paper illustrates that turbidity does not uniformly effect all species of fish the same way, it did point out that the ayu and anchovy, which benefitted from moderate turbidity, were the two species native to traditionally turbid habitats. Yellowtail on the other hand, and open water fish, was only detrimentally effected by the turbidity. This suggests that the yellow eyed mullet, a fish which regularly encounters turbidity, is very possibly adapted deal with turbidity up to a certain point, though like the ayu and Japanese anchovy, there will also likely come a point where any further increase in turbidity is solely detrimental to schooling.

A finally possibility to explain the lack of change in grouping behaviour is that the group size (4) was too small for the mullet to perform normal grouping behaviour, as mullet tend to school in large numbers. This is purely speculator, but could easily be tested by repeating this experiment or similar with larger numbers.

4.42 Response to a simulated predator

At no turbidity did mullet show a decrease in activity immediately following a simulated predator, though one is hinted at 10 NTU. This looks to mean that mullet only show a response to a simulated aerial predator in clear water. This could be interpreted in a number of ways. It could possibly mean

that only at 10 NTU did the rapid dimming and brightening of the lights register with the mullet as a possible predator. This seems unlikely however, as the sudden decrease and increase in lighting would be obvious even at higher turbidities, albeit the change may not seem as extreme as the full brightness of the lights would be reduced due to shading from the suspended sediment (Benfield and Minello, 1996).

Another possibility is that at higher turbidities mullet can detect the predator stimulus, but have no need to respond because turbidity provides them with ample cover from the aerial predators of their native ranges. Sonar data taken from ongoing research at Plant and Food Research feeding station in Nelson, New Zealand shows that aerial shag strikes upon yellow eyed mullet are common at low turbidities, indicating that the avian predators of the yellow eyed mullet may be hindered by turbid water. However Grémillet et al. (2012), a study on great cormorants, *Phalacrocorax carbo*, in Macedonia lake systems, demonstrates that great cormorant populations are unaffected by the turbidity of the lakes they feed from, and that their population size correlates with fish abundance alone.

Further adding evidence to the fact that yellow eyed mullet are not protected from aerial predation by turbidity is Shingles et al. (2005), a study on the response of flathead gray mullet, *Mugil cephalus*, to a model predator. This study describes how gray mullet live in environments that are prone to hypoxia, and to compensate for this, perform a behaviour known as aquatic surface respiration (ASR). Aquatic surface respiration is a behaviour where fish ventilate their gills with water that has a higher oxygen content due to its proximity with the air. Though this behaviour allows the mullet to live in an otherwise anoxic habitat, it does expose them to significant risk of predation by birds. Shingles et al. (2005) found that flathead gray mullet exhibited a fear response of a bradycardia (a rapid slowing of heart rate, generally to conserve oxygen) and initiating hyperventilation when presented with a model avian predator. In addition to this, the mullet, post-predator stimulus, changed their ASR behaviour to surface in more protected locations, such as under shelter and at the edges of the aquarium. When this same procedure was repeated in turbid water (300 NTU) however, the flathead gray mullet exhibited no fear response when presented with the model predator, similar to how the yellow eyed mullet didn't have any apparent response to the simulated predator at any turbidity other than 10 NTU. Furthermore, despite having no reaction to the predator in turbid water, the flathead gray mullet did adopt the alternative ASR behaviour, surfacing in sheltered areas, and did so regardless of whether the predator model was presented at all. This indicates that the flathead mullet were unable to perceive the model predator in turbid water, yet, likely due to their inability to assess predation risk from avian predators when in turbid water, adopted the alternate ASR behaviour, in essence, "playing it safe". If the yellow eyed mullet are

similar to the flathead gray mullet, then it is very possible that they are also unable to detect a predator in turbid water. It is worthy of note however that the experiments with flathead gray mullet were performed using a model bird passing overhead multiple times, whereas the predator stimulus used in the present work was achieved by dimming the lights and bringing them back up quickly, simulating the passing shadow of a bird. It can only be speculated how these two stimuli vary on the response they produce, though one would image that the “passing shadow” stimulus would be more conspicuous than the model at higher turbidities.

In summary, the activity of mullet decreased significantly between 10 and 50NTU and then remained at that low level up to 170 NTU. Grouping behaviour of the mullet however, was unaffected by turbidity. This suggests that during periods of high turbidity, mullet decrease activity perhaps to conserve energy at a time when feeding is likely hampered by turbidity. In light of this implied impairment of vision, the lack of change in the grouping behaviour could be indicative that the mullet rely on other senses, such as olfaction or tactile sense, to maintain grouping in turbid conditions. In terms of predator response, strictly speaking, the mullet display no significant activity response to a simulated predator. However, a response is close to significant at 10 NTU, with the mullet demonstrating an increase in activity immediately following the passing shadow test. In light of another study conducted on another species of mullet, it is very possible that the ability of yellow eyed mullet to perceive an avian predator is constrained by turbidity, adding weight to the argument that, given a larger sample size, the response at 10 NTU may prove to be significant. The experiments presented currently form the basis for further inquiry.

As also suggested in chapter 3, a series of feeding experiments conducted at a range of turbidities will help determine if the reduced activity exhibited by mullet at turbidity is in fact an energy saving mechanism in face of a reduced ability to find and capture prey, or if there is another explanation. Repeating the grouping experiments with a larger test tank and a larger number of mullet in each test group will show whether the present results are representative, or are misleading due to the experimental apparatus not providing enough space for the mullet to properly exhibit any grouping responses they may have. In terms of the response to predators, conducting a similar experiment to that presented in Shingles et al. (2005), but with more intermediate turbidity levels and the exclusion of anoxic conditions. The use of a model predator, as well as physiological monitoring may give a better indication of the fear response of the yellow eyed mullet over using a simple passing shadow test. Looking at the predation of mullet by avian predators in the field is another avenue for further research. Although it would not shed light on the precise response yellow eyed mullet have to perceived avian predators, examination of the number of bird strikes that occur at different turbidities in wild habitat would allow assessment of how at risk the mullet are to predatory birds at

different turbidities. As the results currently stand, it would appear that turbidity has a negative effect on mullet activity (and therefore feeding by proxy), a neutral effect on grouping behaviour, and no significant effect on predator detection, though the data hints at a negative effect is possible. Further work in this area will be crucial to picking apart the causes and significance of these results, and determining the true effect that turbidity has upon the yellow eyed mullet.

5 EFFECT OF TURBIDITY ON THE RESPIRATION OF YELLOW-EYED MULLET (*ALDRICHETTA FORSTERI*)

5.1 INTRODUCTION

Turbidity can affect the respiration of fish in a few different ways. In the field, turbidity of water can be caused by any number of different particulate materials being suspended in the water column. The focus of this chapter is purely the impacts that the physical aspects of turbidity can have on respiration, roughly divided into those of the longer term and shorter term. Firstly, the physical nature of suspended solids means that some can have an irritating or abrasive effect on delicate gill structures, a problem which tends to present itself over a period of time. Secondly, the interaction that these solids have with light means reduced visibility for many species, which for some fish may represent a disadvantageous change in conditions. If a fish interpreted such a change as detrimental, we could see an increase in respiration as a manifestation of stress in response to an acute change in turbidity. This second possibility, a stress induced increase in respiration, is an immediate response that would be measurable in the short term and forms the basis for the investigation in this chapter.

In estuary environments, turbidity can vary over short and long timescales, and acute pulses of suspended sediments are not unusual. However, it is chronic exposure to turbidity that is better studied, in particular the gill damage that can be caused by prolonged exposures to suspended sediments. Au et al. (2004) investigated the effects of prolonged exposure to elevated levels of suspended solids on the green grouper, *Epinephelus coioides*. From 6 weeks of exposure to a range of turbidities (found in wild habitat), the grouper suffered gill damage that correlated strongly with the level of the turbidity. This damage was characterised by epithelial lifting, reduced epithelial volume, and hyperplasia in the pillar system. Irregularities in levels of Na^+, K^+ -ATPase and chloride in the gill lamellae indicated that the fish were experiencing osmoregulatory stress. Although food intake and growth were unchanged by turbidity over the 6 weeks, the results were deemed to show that turbidity of the levels tested caused sub-lethal stress and compromised the health of the fish.

Greer et al. (2015) showed that the oxygen consumption of brown trout, *Salmo trutta*, is unaffected by sediment concentrations of up to 600mg L^{-1}. In Reid et al. (2003) brown trout, *Oncorhynchus mykiss*, were exposed to levels of suspended sediment of approximately 200 - 300mg L^{-1} . Even at these lower concentrations of suspended sediments, the brown trout adopted higher respiration

rates, though there was no damage caused to the gills. Reid et al. described this increased respiration to be in line with an acute stress response to high levels of suspended sediment seen in Coho salmon and steelhead salmon.

Studies extend beyond the effects to commercially relevant fish with elevated turbidity also being shown to cause gill alterations in clownfish larvae, *Amphiprion percula*. Hess et al. (2015) found that when clownfish larvae were experimentally exposed to levels of turbidity which can be found at the Great Barrier Reef (Australia), the gill epithelium of the larvae thickened and produced more mucous. Furthermore, the bacterial communities naturally found in the gills were found to shift from healthy to pathogenic, increasing disease susceptibility. Two types of freshwater minnow, the white tail shiner, *Cyprinella galactura*, and spotfin chub, *Cyprinella monacha*, show similar responses. Sutherland and Meyer (2007) demonstrated that a 21 day exposure elevated to suspended sediment concentrations up to 500 mg L^{-1} caused gill damage and reduced growth rate in these two species. The spotfin chub was most impacted, at the 500 mg L^{-1} concentration showing a 15 fold decrease in growth rate as compared to a control, and severe damage to the gills. As with the clownfish larvae in Hess et al. (2015), epithelial thickening occurred in the gill lamellae. This thickening was found to be inversely related to the growth rate, and thus it is likely that impeded oxygen uptake was a factor in the reduction in growth rate.

Lowe et al. (2015) demonstrates an even more extreme response to turbidity. Part of the study focused on the effects of month long exposure to elevated turbidities on juvenile snapper, *Pagrus auratus*. The turbidity levels used in the study ranges from <10 to 160 NTU. Just as in previous examples, higher turbidities resulted in epithelial hyperplasia and fusion of lamellae. In addition to this, higher rates of the gill operculum opening were detected, indicating higher ventilation rates. In addition to the reduction in growth rate found in minnows by Sutherland and Meyer (2007), Lowe et al. (2015) found that juvenile snapper actually lost weight and in some cases even died due to exposure to prolonged elevated turbidities.

Though Lowe et al. (2015) concludes that reduced oxygen uptake ability due to changes to gill morphology are likely at least responsible for the loss of mass and degradation of snapper health, more recent findings indicate that this is not the case. Cumming and Herbert (2016) was a similar investigation where juvenile snapper were exposed to turbidities between <10 and 80 NTU for 30 days. After this period, the oxygen consumption of the fish was measured and revealed that, contrary to expectations, the oxygen uptake abilities of the fish were undiminished. Cumming and Herbert (2016) speculate that other physiological or behavioural mechanisms could be

compensating for the increased diffusion distances in the gills, or that the modifications to the gills didn't impede oxygen uptake as expected.

Most studies which focus on the impacts of high concentrations of suspended sediment on respiration, look at a long time scale, perhaps influenced by the assumption that gill damage was the main driver or diminishing fish health. Cumming and Herbert (2016) gives reason to believe that, at least in the case of estuarine juvenile snapper, there is no long term impact on respiration rates caused by high turbidity. Overlooked, by the literature in general, is the possibility that acute changes in turbidity may cause changes in respiration in the short term. Although perhaps unlikely to shed light on the causes of physiological stress caused by long term exposure to turbidity, this is an avenue that remains largely unexplored. It is the acute responses to turbidity changes that this research concerns itself with. Acute changes in a number parameters can cause a response in the respiration of a fish which is symptomatic of elevated stress. For example, in Bosch-Belmar et al. (2016) the oxygen consumption of European sea bass, *Dicenrarchus labrax*, was measured under different combinations of temperature, anoxia and jellyfish sting stresses. Oxygen consumption increased under these stresses, which is of particular significance as respiratory distress is expected to interfere with overall metabolism and thus impact the growth and general health of the fish.

As already briefly mentioned in Chapter 1, in some cases, these materials may, through chemical interactions, effect a fish directly, such as by introducing foreign compounds into the body of a fish (e.g. poisons) (Davies-Colley and Smith, 2001). Another common way in which turbidity causing material can disrupt respiration, is by reducing the amount of oxygen in the water. This commonly occurs with eutrophication, where the microorganisms responsible for the turbidity also consume large amounts of the oxygen in the water (Cloern, 2001; Smith et al. 1999).

Turbidity, increased above the range for which mullet are adapted to, may produce an increase in oxygen consumption, perhaps due to the stress of being in a suboptimal environment. Elevated turbidity could also result in a decrease in oxygen consumption on account of the lower activity levels that mullet express in higher turbidity (chapter 4). If mullet oxygen consumption is unchanged by turbidity, that would be a good indication that mullet are likely well adapted to live at a range of turbidities. It are these potential relationships, or perhaps other unpredicted responses to turbidity that this investigation seeks to identify.

5.2 MATERIALS AND METHODS

Experimental setup:

Six mullet were used for these experiments (average weight: 108 g, average length: 230 mm). As depicted in figure 14, respirometry experiments were performed in a 7.25L respirometer. This respirometer was sealed and sat submerged in a water bath. Interfacing with this respirometer were an inlet pipe attached to a pump, an outlet pipe (both of which had manual valves), and an oxygen probe (Firesting robust oxygen dipping probe (OXROB10) which was fitted inside a rubber bung. This oxygen probe was connected to a Firesting unit (Firesting single channel oxygen meter) which measured the partial pressure of oxygen within the respirometer. The data produced by the Firesting oxygen meter was recorded on a laptop using Pyroscience oxygen logger software version 3.213. The other ends of the inlet and outlet pipes both sat within a reservoir from which water could be pumped into the respirometer if the valves were open. It was in this reservoir that the turbidity level was controlled by adding porcelain dust. The experiments were performed within a temperature controlled room set to 15 °C

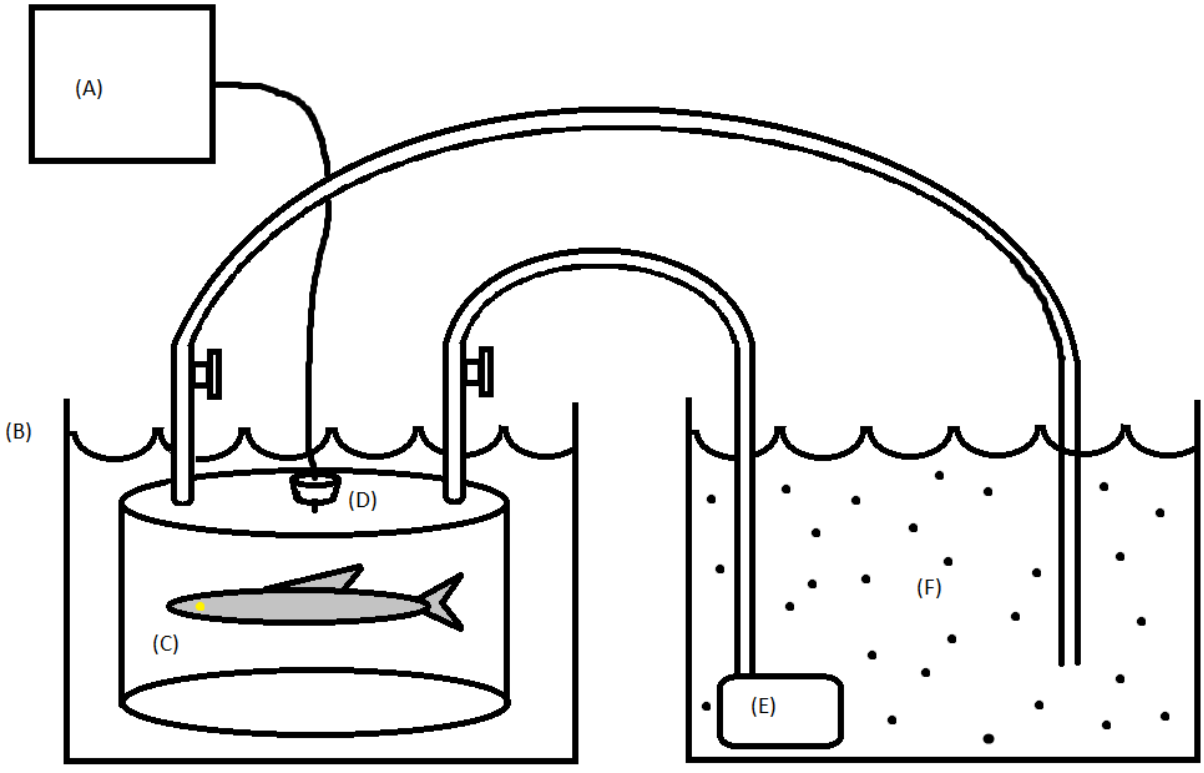

Figure 15 Respirometry setup with Firesting 0_2 sensor, (B) Water bath, (C) Respirometry chamber, (D) Robust O_2 Probe, (E) Pump, (F) Turbid reservoir

Experimental procedure:

Fish were taken from the home tank and were placed into the respirometer the afternoon prior to experimentation (where the light dark cycle of the home tank was maintained), allowing time to acclimate to the experimental setup. At this point the turbidity of the water within the respirometer and reservoir was at the turbidity of the home tank; approximately 10NTU. Experimentation began 17 hours later, with the valves being closed, and the decay of the oxygen content within the respirometer being logged by the Firesting. Oxygen consumption was measured for 20 minutes, or until the partial pressure of oxygen fell below 110mmhg, whichever came first. After this, the valves were reopened, allowing water from the reservoir to flow through the respirometer again and refill it with fully oxygenated (150mmHg) water. This process took approximately 20 minutes, and during this time the turbidity of the reservoir and respirometer was increased to 50 NTU by adding porcelain dust to the water and mixing it in. Once oxygen levels were restored, the valves were closed again, and the oxygen consumption was measured the same as before. This process was repeated, taking the turbidity from 10 NTU and 50 NTU, up through 90, 130 and finally to 170 NTU, giving 5 sets of oxygen consumption data per fish tested.

For statistical analysis, the data was configured into a range-standard-deviation-mean boxplot. Relationships deemed to be worth investigating (i.e. those that appeared to have a chance of being statistically significantly different) had t-tests conducted on them after the assumptions of those t-tests had been met.

5.3 RESULTS

Metabolic rate did not change significantly with turbidity. 10 NTU did not differ significantly from 50 (p-value = 0.8983) and 50 NTU did not differ significantly from 90 NTU (p-value = 0.5068). From assessing the range-standard-deviation-mean boxplot of the data (figure 19), no other relationships were deemed worthy of further analysis.

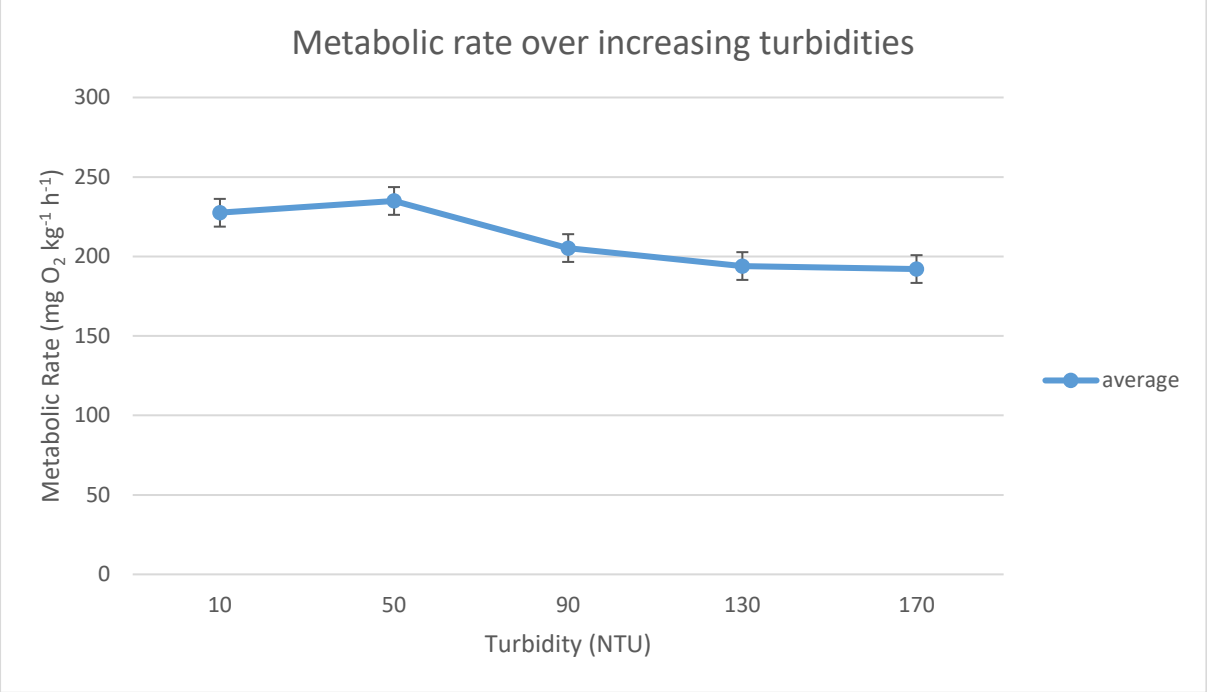

Figure 16 Metabolic rate over a range of turbidities.

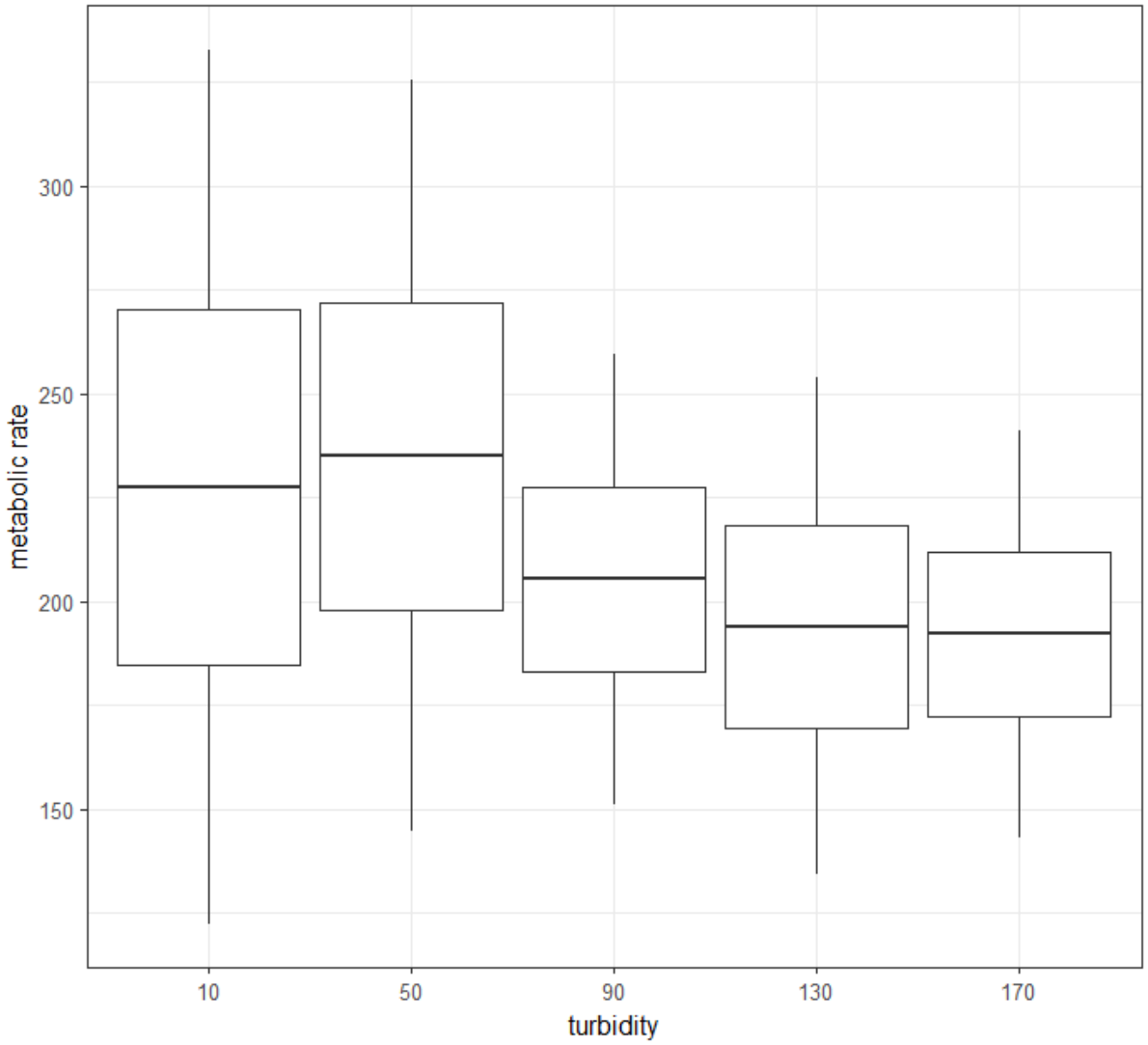

Figure 17 Range-standard-deviation-mean box plot of the metabolic rate of mullet at a range of turbidities.

5.4 DISCUSSION

The respiration of yellow eyed mullet does not change significantly with turbidity over the time scale tested. There is no statistically significant difference between the responses at any of the treatment levels. Preliminary testing also indicated that the respirometry procedure, in absence of changing turbidity levels, did not alone produce a trend in oxygen consumption.

These results are consistent with one of the possible outcomes outlined in section 5.1, where oxygen consumption was not significantly impacted by turbidity, indicating yellow eyed mullet are well adapted to a range of turbidities. A lack of increasing oxygen consumption indicates that an acute increase in turbidity is likely not stressful for a yellow eyed mullet, and the absence of a decrease in oxygen consumption means that either mullet do not decrease metabolism significantly with a reduction in activity (a behaviour seen in chapter 4), or that that approximately 20 minute trials were not long enough to capture the subtle decrease in oxygen consumption resulting from lower activity.

Figure 18 gives the impression that there could be a subtle trend showing decreasing oxygen consumption with increasing turbidity. However, this apparent trend is entirely due to one fish which exhibited far higher respiration than the others at 10 and 50 NTU, dragging up the average response for these treatments. It is uncertain what caused these outliers, though it is possible that due to the fact that the respirometry setup required manual inputs to control water flow, the fish could have been spooked during these adjustments. This explanation is an incomplete one though, as if true, no other fish was effected the same way, nor is it clear why the fish was only frightened by the manual manipulations at those two treatment levels.

On the topic of stressed caused by the process of respirometry, it is of note that the process of respirometry is itself generally accepted to be stressful for fish (Clark et al. 2013). Fish were acclimated overnight in an attempt to mitigate any stress caused by handling and the new environment, but captivity within a foreign and enclosed respirometer would likely be conducive to an exaggerated stress response to any further stimuli, such as the sight of an experimenter or vibrations caused by the manual manipulation of valves. Given that a subtle decrease in respiration (on account of reduced activity) was a possible outcome of this experiment, it should perhaps be considered that the yellow eyed mullet may require a longer acclimation period (though a longer period of time spent constrained within a respirometer could also be counterproductive to reducing stress), and a respirometry setup that allows control of valves remotely. Even with these concerns,

respirometry remains the best option for assessing oxygen consumption, and thus improvements can only be found by making tweaks rather than opting for a different method altogether.

In summary, yellow eyed mullet oxygen consumption does not change as a function of turbidity. This is tentatively indicative that mullet are not stressed by acute changes in turbidity, and the result that mullet respiration does not change after just a few hours of exposure to turbidity is not entirely surprising given that in figures 1 and 2 we can see that turbidity levels fluctuate frequently in the Avon-Heathcote estuary. This is not conclusive however, as we are unable to eliminate the possibility that there could be a slight decrease in respiration at elevated turbidities as a result of the decreased activity found in chapter 4. Future work could use a larger respirometer in order to reduce the stress of confinement, and give longer measurement periods to allow the possibly minor reduction in oxygen consumption due to reduced activity adequate time to manifest itself significantly within the data. Larger sample sizes would also benefit this work, as the ones presented currently were small due to limited availability of fish at the time the experiments were conducted.

6 GENERAL DISCUSSION

The investigations presented in the preceding chapters have added to the extremely limited information that is known about the yellow eyed mullet. The optomotor experiments (chapter 3) revealed that the optomotor response exhibited by the mullet in clear water (10NTU) completely diminishes by the time water turbidity has been increased to 50NTU. This tells us that the visual acuity of mullet are in fact impacted significantly by turbidity levels which regularly occur within the estuary. This impairment of a major sense is not on its own necessarily indicative that turbidity at these levels negatively effects the heath or survival chances of the yellow eyed mullet, but in order for mullet to remain unimpacted by these levels of turbidity, the mullet would need to have other very keen senses, such as olfactory sense and tactile sense, to compensate.

The activity experiments (chapter 4) revealed that the activity level of yellow eyed mullet in clear water 10 NTU reduces significantly with increasing turbidity. Somewhere between 10 and 50 NTU, the activity of the mullet (as measured by tank sections crossed in a given amount of time) dropped to a base level which was maintained from 50 to 170 NTU. From the literature, we learn that the main reason reported to explain decreased activity in turbidity is that turbidity decreases an organism's ability to locate and capture prey items. By lowering activity in turbid conditions, mullet are able to conserve energy that would be otherwise wasted in a futile pursuit of food. This explanation implies that mullet do in fact primarily rely on vision as a means of locating and catching food items, as predators which locate food through primarily through tactile or olfactory means are largely unaffected by turbidity. This ties in nicely with the reduced visual acuity found in chapter 3, showing that both visual acuity and activity decrease over the 10 to 50 NTU range.

The grouping experiments (chapter 4) revealed that the grouping behaviour of mullet was unaffected by turbidity. No significant difference was found between the grouping exhibited at any of the turbidity levels tested. If these results are representative of how mullet behave in the wild, then vision is not as important as other factors in how the mullet maintain their nearest neighbour distance. It is very possible that mullet predominantly rely on tactile information gathered via the lateral line, and/or rely on olfactory information to help them remain aggregated. However, it is also possible that this experiment was not suitably designed to properly assess the grouping behaviour of mullet. Mullet are quite fast swimmers, and are known to school in large numbers. These grouping experiments were conducted in a relatively small tank, only 1.5 m in diameter, and only with a small number of fish. It is conceivable that in the wild where mullet are in greater numbers, are able to

travel at higher speeds, and are able to traverse much greater distances, they may struggle to stay together in elevated turbidity.

Like the grouping experiments, the predator response experiments did not find a significant result. There was no statistically significant difference between the responses of the mullet at any of the turbidities tested. There was a visible response immediately following the passing shadow test at 10 NTU, which though statistically insignificant, hints that there could be a response here. If this is the case, it is backed up by the fact that flathead gray mullet have been shown to exhibit a response to a model avian predator in clear water, and the fact that Plant and Food Research in nelson has sonar recordings that indicate bird strikes are common at lower turbidities. Though mullet may find it more difficult to detect avian predators at higher turbidities, it is also true that the avian predators themselves may struggle to accurately detect the mullet. Research shows that at least some avian fish predators appear not to be effected by the moderate levels of turbidity when hunting fish, though whether this can be said about the avian predators of yellow eyed mullet remains to be seen.

Finally the respirometry experiments showed that there is no significant change in the oxygen consumption of yellow eyed mullet at any of the turbidities tested. In isolation, this could lead us to believe that acute changes in turbidity do not cause mullet any amount of stress detectable via oxygen consumption, and therefore conclude that mullet are well adapted to deal with this range of turbidities. While this could be the case, the other findings presented here suggest that the mullet are not in fact equipped to deal with turbidities over 50 NTU. It seems unusual that the respiration of the mullet is unchanged, as we have seen that activity decreases with increasing turbidity, and thus we would expect oxygen consumption to decrease too. If this hypothesis is correct, then we can conclude that the effect may have been too small to detect in these experiments, possibly because 20 minute windows of recorded oxygen consumption may not have been long enough, and possibly because stress from the respirometry procedure masked the effect.

Taken together, these experiments are the beginnings of demonstrating that yellow eyed mullet are likely detrimentally impacted by the high levels of turbidity that can be found with the Avon-Heathcote estuary. If this is in fact the case, this is a problem for yellow eyed mullet, as the Avon-Heathcote estuary is not alone in experiencing great fluctuations in turbidity which are exacerbated or entirely created by human activities. Although mullet are able to tolerate a large range of environments and salinities (being found in rivers, estuaries and coastal waters) and this may allow them to escape habitats as turbidity gets too high, they ultimately must be able to tolerate the

extremes of the likes of Avon-Heathcote estuary as this is thought to be an important breeding ground for the yellow eyed mullet population in the waters surrounding Christchurch, New Zealand.

Information on how turbidity effects the predation risk upon mullet remains unclear. In order to clarify this relationship, field work observing the frequency of avian predators attacking mullet at different turbidites would add greatly to our understanding of the disadvantages or benefits that turbidity provides mullet in the context as prey. Also any information on what aquatic predators prey on mullet would be useful, so the performance of these species could be more closely examined so as to assess their ability to prey on mullet under different turbidity levels.

Across each of these experiments, 50 NTU appeared several times as the turbidity at which the measured response of the mullet changed. Because there were no sampling points between 10 and 50 NTU, all that can be said is that somewhere between these two turbidites, there appears to be a turbidity level which is biologically significant to yellow eyed mullet. Further investigation should explore the 10 to 50 NTU range with higher resolution in order to locate this biologically significant level. It is also of note of the studies presented here deal with the response of the yellow eyed mullet to an acute change in turbidity. In order to gain a fuller understanding of the potential risk that turbidity poses for the species, the response of mullet to chronic turbidity should be recorded for comparison with this study, and chronic turbidity studies in other species.

For now all we can say is that turbidities above 50 NTU appear to have a significant impact on the lifestyle of the yellow eyed mullet. This is important in a management context, as although Avon-Heathcote turbidity tends to be under 50 NTU, periods where this turbidity level is exceeded are not uncommon. If turbidity fluctuations worsen in the future as the city of Christchurch and its surrounding areas continue to develop and degrade the water, yellow eyed mullet in the area could be at risk which is significant from both a conservation and fisheries perspective.

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